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[NFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON INFECTION OF WHEAT SEEDLINGS BY HELMINTHOSPORIUM SATIVUM [1]

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INTRODUCTION

While it is not the purpose of this paper to deal with all phases of the Helminthosporium disease of wheat, it seems advisable at this time to ummarize the general situation concerning the disease. The chief purpose of this paper is to present the results of field observations and preliminary experiments bearing on the influence of soil temperature and soil moisture on certain phases of seedling infection in spring and winter wheat and, to a limited extent, in spring barley.

When the writer began the investigation of the take-all and the rosette liseases of wheat it became evident that these diseases were in many ases intimately associated with the Helminthosporium disease and also with other wheat diseases which were likewise obscure. This necessitated a study of certain phases of the Helminthosporium disease in order that he other maladies might be properly interpreted.

Although the Helminthosporium disease of wheat had attracted little attention among plant pathologists prior to the discovery of the rosette lisease of wheat in Madison County, Ill., in 1919 (*11*),[3] it was known to occur in several of the spring-wheat States and, to a limited extent, in the winter-wheat area. While little had been published in connection with the Helminthosporium disease, cereal pathologists in the spring-wheat belt and adjacent areas were fairly familiar with its general symptoms and characteristics.

Beckwith (*1*) and Bolley (*2*) were the first to show that wheat plants may be attacked by a Helminthosporium and that this organism is ssociated with poor wheat yields in the spring-wheat area. E. C. ohnson (*6*) was the first to demonstrate the pathogenicity of Helminhosporium on wheat seedlings. While he called the species with which e worked *Helminthosporium gramineum* Rabh., it is evident from the ehavior of his fungus in inoculation experiments that, in reality, he was orking with *H. sativum* P. K. and B.

[1] Accepted for publication May 2, 1923. The greenhouse and laboratory studies reported in this paper re carried on cooperatively between the Office of Cereal Investigations, U. S. Department of Agriculture, d the Wisconsin Agricultural Experiment Station, Madison, Wis. The field studies were conducted ar Granite City, Ill., in cooperation with the Illinois Agricultural Experiment Station in connection th the investigations of the rosette disease of wheat.

[2] The writer wishes to express his appreciation to Prof. L. R. Jones and Dr. A. G. Johnson for the many ggestions concerning the work herein reported, and to Mr. R. W. Leukel for assistance in conducting e greenhouse experiments.

[3] Reference is made by number (italic) to "Literature cited," p. 217.

Soon after the discovery of the rosette disease (*11*) near Granite City, Ill., and in Indiana, it was found by the writer and others that *Helminthosporium sativum* P. K. and B. was associated with it in its later stages. While this association was rather consistent in many cases, it seemed somewhat doubtful to the writer and to certain other workers if Helminthosporium was to be looked upon as the primary cause of rosette, although it was recognized that rosette might possibly be an unusual manifestation of the Helminthosporium disease (*9*) brought about by some environmental condition, or due to some unfamiliar strain of the organism. Although Stevens (*14, 15*) claims to have proved that the rosette disease (called footrot by him) (*13, 14, 15*) is caused directly by Helminthosporium, it has been pointed out by the writer (*9, 11*) that positive proof of this causative relation is lacking. As to the ability of Helminthosporium to produce a definite, unmistakable disease in wheat, there is no doubt, as is shown in Plates 1, 2, 3, and 4, but as to its ability to produce the symptoms of wheat rosette, as the latter malady is now interpreted, there is a question.

On a basis of field observations and isolations made from material collected by the writer and others, it is evident that the Helminthosporium disease of wheat occurs to a greater or less extent throughout the wheat-growing regions of this country (*10*).

In some cases the disease occurs in combination with other wheat diseases, as is the case in the district around Granite City, Ill., and in certain of the fields affected by take-all and footrot in Kansas. In other cases *Helminthosporium sativum* seems to be the chief or only parasite involved.

In certain localities and under certain conditions the Helminthosporium disease causes considerable damage to the wheat crop. This is evidenced by the conclusions of Bolley (*3*) and Stakman (*12*) concerning Helminthosporium in North Dakota and Minnesota, respectively, and of Hungerford (*5*) concerning the situation in the vicinity of Rexburg, Idaho, in 1921.

While more than one species of Helminthosporium may be involved in the disease, the bulk of evidence now in hand, as pointed out by the writer (*10*), indicates that a single species (*H. sativum*) is the one chiefly involved. This organism apparently does not have as fixed a morphology as many fungi, and this is especially true in regard to conidia. Experiments which will be discussed fully in a later paper show that the same single-spore isolation, when submitted to different conditions as to substratum, temperature, etc., may produce spore forms which are so widely different as to suggest different species to persons not acquainted with the situation. As to the possibility of different physiological strains within this species nothing definite can be said at this time.

The studies on the symptoms of the Helminthosporium disease as published by Stakman (*12*) and by the writer (*11*) show that under favorable conditions *H. sativum* is capable of attacking all parts of the wheat plant from the roots to the head. It is evident, however, that under certain conditions infection does not take place, or takes place only in a mild form, even when the organism is present in the soil.

Soon after the writer became interested in the Helminthosporium disease, it was realized that the disease does not attack the wheat plant with the same degree of severity in all localities or during the different periods of development of the plant in a given locality. As pointed out earlier in a brief note (*10*), these observations led to the

belief that climatic factors and weather conditions probably exerted some influence on the development of the disease. Accordingly, laboratory and field experiments were planned whereby data on these influences might be obtained. Since *H. sativum* attacks all parts of the plant it is obvious that the different types of injury should be studied more or less independently. In view of this fact it was decided to make the preliminary studies on those injuries which are confined to the subterranean parts of the plant, and on the development of these injuries as influenced by soil temperature and soil moisture.

GREENHOUSE EXPERIMENTS

SOIL TEMPERATURE STUDIES

All of these studies were carried out in the department of plant pathology, University of Wisconsin. The soil-temperature apparatus used was essentially the same, except for some modification, as that described by Jones (7).

The wheat seedlings were grown in metal pots 8 inches in diameter and 9½ inches deep, placed in tanks of water held at the desired temperatures. The water line came from ½ to 1 inch above the soil line in the pots. Previous experimeuts with potatoes in connection with the soil-temperature studies on potato scab by Jones, McKinney, and Fellows (8), and also preliminary experiments with wheat plants, showed that there was no need for drainage in the metal pots, and, therefore, no special drainage apparatus was used.

EXPERIMENTS AT CONSTANT TEMPERATURES

Experimental Methods

The various temperatures were maintained by electric heaters placed on the bottoms of the tanks in contact with the water, and by means of cold running water supplied from the local mains in winter and from a refrigeration coil in summer. The high temperatures were controlled by electric thermostats which opened and closed the heater circuits by means of relays. These regulated to within an average of ½° to ¾° C., above and below the stated temperature. The low temperatures were regulated by carefully adjusting the inflow of cold water or by a controlled electric heater which operated against a stream of cold water having an inflow slightly greater than that required to hold the proper temperature in the soil. All temperatures were regulated and recorded on a basis of the temperature of the soil 1 inch below the surface and 1½ inches from the walls of the pots.

All plants were watered on a basis of weight with tap water frequently enough to insure a nearly constant soil moisture throughout an experiment. At the high temperatures pots were watered daily or oftener, depending upon the weather, while at lower temperatures the watering was less frequent. Different methods have been used, but in this work it seemed that the application of water directly to the surface of the soil was best when watering was done frequently. In all the experiments, the plants in a given soil-temperature series were subjected to the same air temperatures, which ranged from approximately 18° to 24° C., according to the season. The differences in host response and the development of disease were due, therefore, primarily to differences in soil temperature.

All soil used in the soil-temperature studies consisted of a fertile loam obtained from a wood lot. Although this soil had never been cropped it

was infested with *Helminthosporium sativum*, which develops on many of the wild grasses. This necessitated sterilizing the soil by the pressure-steam method for varying periods, depending on the pressure used. Four hours at 1-pound pressure or less and one hour at 10 to 15 pounds gave satisfactory results. This soil after sterilization had a moisture-holding capacity of 67 per cent. Two varieties of wheat, Marquis (spring) and Harvest Queen (winter), and Hannchen and Hanna varieties of spring barley were used in these experiments. All seed was surface sterilized with a solution of mercuric chlorid and water (1:1,000) for 10 minutes and thoroughly rinsed in sterile water before sowing. It was very difficult to obtain seed free from Helminthosporium infection and, as surface sterilization is not effective in controlling this infection, such seed had to be guarded against. One sample of Harvest Queen seed from the uplands of Madison County, Ill., was for the most part free from infection, and this was used in much of the work. A small amount of Marquis seed, kindly supplied by G. H. Dungan, of the Illinois Agricultural Experiment Station, also proved to be practically free from infection, and the same was true of the seed of Hannchen and Hanna barley from the Aberdeen (Idaho) plots of Dr. H. V. Harlan, of the Office of Cereal Investigations, United States Department of Agriculture.

The organisms used in the inoculations consisted of three single-spore strains of *Helminthosporium sativum*. The first, designated No. 51a, was isolated by the writer in May, 1920, from the crown of a Harvest Queen wheat plant, in the advanced stages of the rosette disease, growing near Granite City, Ill. The second, designated No. 350, was isolated by the writer in April, 1921, from an infected barley kernel obtained from a lot of seed grown in the vicinity of La Fayette, Ind. The third, designated No. 392, was isolated by Dr. R. W. Webb in the spring of 1921 from the same type of plant and from the same source as culture 51a.

These strains were cultured on potato-glucose agar in Petri dishes. The spores were scraped from the surface of the medium and put into water. These spore suspensions were then used to inoculate the seed or the soil before sowing.

In the case of seed inoculation, a given volume of spore suspension was placed in a test tube, such volume being just enough to moisten the number of seeds to be sown in a single pot. The suspension was carefully measured by means of a pipette so as to insure uniformity of inoculation and then put into as many test tubes as there were pots to be inoculated. This measuring procedure was followed at the beginning of the inoculating operation. The seeds were previously counted out in definite numbers for each pot. At the time of sowing a particular pot, the seed was poured into the test tube of inoculum, well shaken, and emptied into a Petri dish, the small excess of suspension was drained off, and the seeds were quickly planted by means of forceps. Seeds were not introduced into the inoculum until just before planting. All seed was sown 1.5 inches deep.

Owing to the fact that the spores of *Helminthosporium sativum* do not germinate to any extent in large quantities of water, no bad effects come from preparing all of the suspensions at the beginning of the sowing operations. The writer has had a spore suspension of this organism in the laboratory from April to November, 1921, with practically no germination. Sowings of these spores were made on potato-glucose agar from time to time, and good germination took place until the latter part of the period, when the viability of the spores seemed to go down rapidly.

Soil inoculations were made by sprinkling or spraying a spore suspension over all the soil used in a complete series. This soil afterwards was thoroughly mixed and put in the pots before the seeds were sown. This method insured uniformity of the inoculum throughout all the pots in a series. In all cases the control or uninoculated pots were sown before working with the inoculum for the inoculated pots.

In all of the experiments, only enough inoculum was used to produce a moderate amount of infection on the underground parts. This was done in order that the temperature influence might be determined more accurately. In no case was there sufficient inoculum to produce a marked killing of the plants. In the soil-temperature studies on potato scab (*8*) it was found that heavy inoculation tended to produce undue flattening of the temperature and disease curve, and this same condition seems to hold with the Helminthosporium disease. The exact temperature optimum tends to be obscured when an excess of organism is present.

In determining the comparative influences of the several soil temperatures in any one series the amount of disease produced was taken as a basis. As pointed out in the work with potato scab (*8*), it is not adequate to use alone either the number of infected individuals or the degree of infection as the sole index for the amount of disease.

In the case of the data from the greenhouse experiments the extent of disease is expressed as an infection rating, which represents the percentage of the total number of plants which were infected and also the degree of infection.

In recording the extent of disease, the plants were separated into five classes according to the degree of infection, and each plant was given a numerical rating, as shown in Table I.

TABLE I.—*Classes, degrees of infection, and numerical ratings used in rating diseased and healthy wheat*

Class.	Degree of infection on the underground parts.	Numerical rating.
1	None	0.00
2	Very slight	.75
3	Slight	1.00
4	Moderate	2.00
5	Abundant	3.00

The classes are described as follows: (1) No signs of infection, as evidenced by the absence of any lesions on the underground parts; (2) very light infection, as evidenced by small lesions on the coleoptile; (3) slight infection, as evidenced by small lesions on the coleoptile or sheaths in excess of (2); (4) moderate infection, as evidenced by the partial or almost complete rotting of the coleoptile, with a few lesions on lower leaf sheaths or roots; (5) abundant infection, as evidenced by a complete rotting of the coleoptile and numerous lesions on the subcrown internode[4], lower leaf sheaths or roots.

In most of the experiments herein cited relatively slight root infection occurred. Whether this is due to a difference in resistance between the

[4] The term subcrown internode is here used to apply to the elongated structure of the wheat plant which, under certain conditions, develops between the germinated seed and the crown. In wheat and barley this structure is covered by the coleoptile.

roots and the other underground parts or to some other factor is not known. Further study is being made to determine this point.

After each plant in a given series had been given a numerical rating, the final infection rating for the plants grown at a given temperature was arrived at by adding together all the numerical ratings, dividing this sum by the total number of inoculated plants involved multiplied by three. This result was then multiplied by 100, thus putting the infection rating on a percentage basis.

$$\frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of inoculated plants} \times 3} = \text{Infection rating.}$$

This result is then the comparative infection rating for the given temperature, since three times the total number of plants (3 being the highest numerical rating) represents the highest possibility for disease under the conditions of the experiment. The results from all plants grown at all the temperatures in a given series are compared on a basis of factors derived according to the above method for each separate temperature.

In cases where some Helminthosporium infection occurred in the controls, the number of such infected plants was deducted proportionally from the total number of inoculated plants before determining the disease factor in the inoculated series. Usually the uninoculated control plants were free from infection, but it was found to be very difficult to prevent all contamination, because of the fact that *H. sativum* sporulates so freely.

Results

Host development.—While the experiments cited were designed primarily to yield data concerning the development of the disease, it has been possible also to obtain information concerning the influence of soil temperature on the host plant.

As shown by Dickson (4) and other workers, the host plants react to soil temperature in many respects. In the case of the time required for the seed to germinate and emerge from the soil, this study shows that the higher temperatures, from 24° to 34.5° C., speed up this process in wheat and barley. At 28° emergence takes place in about three days, with 32°, 34.5°, and also 24°, coming on in about four days. At soil temperatures of 20°, 16°, 12°, and 8°, emergence takes place at intervals of about 5.5, 7.5, 10, and 16 days, respectively, from the date of planting.

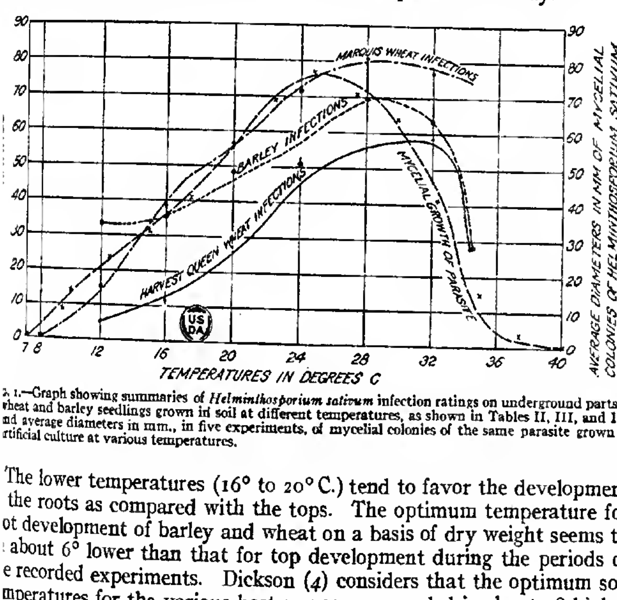
Considerable influence of temperature on the development of the plants after emergence also was found. During the periods of the experiments it was discovered that the greatest development in stature and dry weight of plants took place at temperatures of from 20° to 24° C. This temperature range forms the rather broad crest of a curve which descends gradually toward the higher and lower temperatures.

At 8° C. germination was slow, but a fairly high percentage of seeds germinated. The percentage of germination seemed to be highest at 12°, 16°, and 20°. Owing to the slow development of plants and the slight extent of disease at 8° this soil temperature was not used after the second experiment with Marquis wheat. It was found that very few plants developed at soil temperatures above 35°, and this temperature proved impracticable for the disease experiments. Even at 34.5° there was poor germination and the plants did not thrive.

It was found that a temperature of 20° C. tends to produce the greatest number of tillers in wheat. In 57 days the production of tillers per plant

t 20° averaged 6.2, while at the extreme soil temperatures only two llers per plant were formed. At temperatures between 20° C and both igher and lower extremes a gradual decrease in number of tillers 'as noted.

Under certain conditions with wheat the soil temperature seems to ıfluence the ultimate position of the crown and permanent root system ith respect to the seed and soil surface. At high temperatures the own tends to be developed near the surface of the ground, or, in other ords, a long subcoronal internode is formed; whereas, at low soil mperatures, the crown tends to form low or at the seed. Intergrading lations of these structures develop at the intervening temperatures. ıst how important soil temperature is in connection with this modifiıtion is not known. It seems apparent, however, that other factors ıay, under certain conditions, completely obscure the temperature inıence, for the writer has occasionally observed plants in well-controlled mperature experiments which did not conform to the above observaon. Certain varieties also do not seem to respond in this way.



ı. 1.—Graph showing summaries of *Helminthosporium sativum* infection ratings on underground parts of vheat and barley seedlings grown in soil at different temperatures, as shown in Tables II, III, and IV, nd average diameters in mm., in five experiments, of mycelial colonies of the same parasite grown in rtificial culture at various temperatures.

The lower temperatures (16° to 20° C.) tend to favor the development the roots as compared with the tops. The optimum temperature for ot development of barley and wheat on a basis of dry weight seems to about 6° lower than that for top development during the periods of e recorded experiments. Dickson (4) considers that the optimum soil mperatures for the various host responses recorded is about 4° higher r Marquis wheat than for Turkey. While there is a slight indication this research that this relation holds between Marquis and Harvest ıeen, the evidence is not sufficiently striking to warrant a definite ıtement at this time.

Disease development.—The results of disease development at the veral soil temperatures are tabulated in Tables II, III, and IV. The erage data from all the experiments are tabulated at the end of each these tables and are shown graphically in figure 1. From the several bulations it will be noted that while there has been a slight shifting of optimum soil temperature for disease occurrence in the several eriments, this shifting has been within rather restricted limits.

TABLE II.—*Effects of soil temperatures on the infection of Marquis (spring) wheat seedlings with Helminthosporium sativum, Culture 51a, at Madison, Wis., in 1920–21*

Experiment 1.			Experiment 2.			Experiment 3.		
Artificially inoculated soil. Started Nov. 24, 1920; ended Jan. 20, 1921. Soil moisture 37.3 per cent of moisture-holding capacity.			Artificially inoculated soil. Started Feb. 7, 1921; ended Feb. 23, Mar. 12 and 18, 1921. Soil moisture 44.4 per cent of moisture-holding capacity.			Artificially inoculated seed. Started Mar. 3, 1921; ended Mar. 21, 1921. Soil moisture 43.2 per cent of moisture-holding capacity.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°C.			°C.			°C.		
8	40	1.3	[a] 8	72	24.6	16	103	32.6
12	41	21.1	[b] 12	72	61.0	20	100	73.4
16	37	63.0	[c] 16	73	40.8	24	92	87.7
20	33	73.7	[c] 20	78	52.3	28	90	95.5
24	25	68.0	[c] 24	79	76.3	32	70	79.8
28	22	69.6	[c] 28	71	82.1	34.5	54	58.0
32	20	61.3	[c] 32	27	87.6			
			[c] 35	27	88.8			

Experiment 4.			Experiment 5.			Summary.[d]		
Artificially inoculated seed. Started Nov. 19, 1921; ended Dec. 17, 1921. Soil moisture 59.7 per cent of moisture-holding capacity.			Naturally infected loam soil. Started Nov. 19, 1921; ended Dec. 17, 1921. Soil moisture 59.7 per cent of moisture-holding capacity.			Average amount of infection at each soil temperature in five experiments with Marquis (spring) wheat.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Average number plants per experiment.	Infection rating.
°C.			°C.			°C.		
						8	56.0	1.3
12	82	17.1	12	98	7.9	12	73.2	15.4
16	74	33.3	16	94	25.2	16	76.2	38.9
20	63	46.9	20	74	36.2	20	69.6	56.9
24	67	64.7	24	86	60.9	24	69.8	71.5
28	44	94.2	28	71	64.0	28	59.6	81.[illegible]
32	67	83.4	32	58	75.2	32	48.4	77.4
34.5	41	86.7	34.5	45	73.3	34.5–35.0	41.7	76.7

[a] Ended Mar. 18.
[b] Ended Mar. 12.
[c] Ended Feb. 23.
[d] This summary does not include the 8° or 12° temperature data from experiment 2, since these are for older plants.

TABLE III.—*Effects of soil temperatures on the infection of Harvest Queen (winter) wheat seedlings with Helminthosporium sativum cultures No. 51a and 392, at Madison, Wis., in 1921 and 1922*

Experiment 1.			Experiment 2.			Experiment 3A.			Experiment 3B.		
Artificially inoculated soil (culture 51a). Started Feb. 7, 1921; ended Feb. 23, Mar. 12 and 18, 1921. Soil moisture 44.4 per cent of moisture-holding capacity.			Artificially inoculated seed (culture 51a). Started Mar. 3, 1921; ended Mar. 21, 1921. Soil moisture 43.2 per cent of moisture-holding capacity.			Seed inoculated with a water suspension of culture 51a containing 105,200 conidia per cc.; started Apr. 3, 1921; ended Apr. 27, 1921. Soil moisture 33.5 per cent of moisture-holding capacity.			This experiment was carried on at the same time and in the same manner as experiment 3A except that the seeds were inoculated in a water suspension containing 6,575 conidia per cc.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°C.			°C.			°C.			°C.		
8	94	24.7	16	117	5.5	12	104	7.2	12	[d] 58	0.6
12	84	36.6	20	117	31.2	16	118	12.2	16	118	15.2
16	103	21.0	24	109	75.2	20	118	46.8	20	116	22.7
20	94	31.5	28	112	73.8	24	[d] 55	43.0	24	116	41.6
24	97	31.0	32	114	69.0	28	115	70.7	28	118	46.0
28	88	48.6	34.5	98	41.1	32	101	79.2	32	109	48.6
32	78	74.9				34.5	[d] 79	67.9	34.5	105	33.9
35	70	54.7									

Experiment 4.			Experiment 5.			Experiment 6.			Summary.[f]		
Artificially inoculated seed (culture 51a). Started Apr. 29, 1921; ended May 21, 1921. Soil moisture 32.8 per cent of moisture-holding capacity.			Consolidated temperature data from combined soil temperature and moisture series. Moisture data given in Table VII. Artificially inoculated seed (culture 392). Started May 4, 1922; ended May 26, 1922.			Artificially inoculated soil (culture 392). Started May 20, 1922, ended June 2, 1922. Soil moisture 52.2 per cent of moisture-holding capacity.			Average amount of infection at each soil temperature in the six experiments with Harvest Queen (winter) wheat seedlings.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°C.			°C.			°C.			°C.		
2	99	0.0	12	167	16.5	12	117	6.2	12	109	5.3
6	99	.8	16	175	19.5	16	121	14.5	16	121	10.8
0	56	13.2	20	170	55.3	20	117	17.0	20	112	27.3
4	32	48.5	24	167	71.7	24	117	29.8	24	99	47.0
8	48	47.9	28	167	81.2	28	117	53.6	28	109	55.2
2	24	26.3	32	174	54.5	32	101	79.3	32	100	59.1
4.5	59	9.6	34.5	156	8.7	34.5	[e] 55	64.0	34.5–35	88	28.6

[a] Ended Mar. 18.
[b] Ended Mar. 12.
[c] Ended Feb. 23.
[d] Stand reduced by mice.
[e] Plants in one pot lost during experiment on account of leak in pot.
[f] This summary does not include the 8° or 12° temperature data from experiment 1, since these are for older plants.

TABLE IV.—*Effects of soil temperatures on the infection of Hannchen and Hanna barley seedlings with Helminthosporium sativum cultures 51a and 350, at Madison, Wis., in 1921*

Experiment 1.			Experiment 2.			Experiment 3.			Summary.[e]		
Artificially inoculated soil (culture 51a). Hannchen barley seed used. Started Feb. 7, 1921; ended Feb. 23, Mar. 12 and 18, 1921. Soil moisture 44.4 per cent of moisture-holding capacity.			Artificially inoculated Hanna barley seed, culture 51a used. Started Mar. 3, 1921; ended Mar. 21, 1921. Soil moisture 43.2 per cent of moisture-holding capacity.			Artificially inoculated Hanna barley seed, culture 350 used. Started Apr. 29, 1921; ended May 21, 1921. Soil moisture 32.8 per cent of moisture-holding capacity.			Average amount of infection at each soil temperature in three experiments with Hannchen and Hanna barley seedlings.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temparetures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°C.			°C.			°C.			°C.		
[a] 8	90	24.4	16	110	20.6	12	47	33.0	12	47	33.
[b] 12	89	38.2	20	77	34.3	16	[d] 17	49.0	16	74	35.
[c] 16	94	36.1	24	102	44.6	20	37	69.0	20	69	49.
[c] 20	93	44.2	28	91	72.3	24	28	63.0	24	74	51.
[c] 24	92	46.4	32	88	53.0	28	13	82.0	28	62	69.
[c] 28	83	55.6	34.5	55	30.0	32	14	83.3	32	51	63.
[c] 32	51	54.8				34.5	6	16.6	34.5–35	22	29.
[c] 35	6	41.6									

[a] Ended Mar. 18.
[b] Ended Mar. 12.
[c] Ended Feb. 23.
[d] Number of seedlings reduced due to ravages of mice.
[e] This summary does not include the 8° or the 12° temperature data from experiment 1, since these are for older plants.

While the results of these experiments show that the Helminthosporium disease can develop at all of the soil temperatures employed, they also indicate that the disease is not favored by either relatively high or relatively low temperatures. From the curves shown in figure 1 it is strikingly evident that rather high soil temperatures (28° to 32° C.) favor the development of the disease on the underground parts of the plants during the early period of their development. Although the exact explanation of this result can not be given at this time, it should be noted that the disease temperature optimum is above that for the best development of the host plants and also above that for the best vegetative growth of the parasite in pure culture, as is shown in figure 1. This relation suggests that the relatively high temperature requirements for the best development of the parasite (24° to 28°) together with the probable weakening of the hosts (host optimum 20° to 24°) at such temperatures partially explain the high optima (28° to 32°) for the development of the disease. It should also be noted that the optimum temperature is apparently 4° higher in the case of Harvest Queen wheat than in the case of Marquis wheat or the barleys. The same tendency is suggested in the data published by Dickson (*4*) on the Fusarium seedling blight of wheat, except that he reports lower optima. The explanation of these relations may be tied up with differences in varietal susceptibility or with a number of other unanalyzed factors.

In the results from experiment 2 in Table II, experiment 1 in Table III, and experiment 1 in Table IV, it will be noted that the plants grown at 8° and 12° C. were not removed at the same time as those grown at the higher temperatures. They were removed at later dates for the purpose of getting some idea of the influence of time on the development of the disease. The data recorded in the above table show that time is an important factor, as evidenced by the sharp rise in the disease curve at 8° and 12°, in contrast with the depression of the curves at the low temperature end of the experiments, where plants grown at all temperatures are removed and examined at the same time. These results are in line with natural expectations.

In experiments 3A and 3B with Harvest Queen wheat 105,000 and 575 conidia of the parasite, respectively, per cc. of water were used to inoculate the seed before sowing. The results of this experiment show clearly that the amount of inoculum greatly influences the disease development. In this experiment the greatest amount of disease occurred where the greatest number of conidia were used.

It will be noted in figure 1 that the disease curve for Marquis wheat is considerably higher than those for Harvest Queen wheat and barley, except below 16° C. for barley. This relation is explained for the present on the basis of varietal susceptibility. In all of the work done by the writer to date, Marquis wheat has shown higher susceptibility than barley or the other varieties of wheat used. The indications are that the varieties of barley used develop a greater amount of Helminthosporium infection at low soil temperatures than is the case with wheat; and Marquis (spring) wheat seems to show the same tendency as compared with Harvest Queen (winter) wheat. While these relations seem to be tied up with specific and varietal differences, such a general explanation falls far short of completely satisfying the many questions which come to the mind of the experimenter. It is hoped that more satisfactory explanations for some of these results may develop from research now under way.

EXPERIMENTS AT ALTERNATING TEMPERATURES

Experimental Methods

As far as known, all of the controlled soil temperature studies on plant disease development thus far have had to do with "constant" temperatures. While such temperatures are a means of obtaining very valuable data which may be analyzed readily, it is recognized that under no circumstances in nature is the plant or the disease-producing organism submitted to a constant soil temperature for any length of time. Naturally this may lead some to inquire as to the actual value of constant temperature results as an aid in interpreting the reaction of disease to variable temperatures under field conditions. We are inclined to assume that the average daily soil temperature over a given period will produce practically the same results as a constant soil temperature equivalent to the mean for such a variable. In the case of potato scab this conception seems to hold, as is evidenced by the field experiment and observations on soil temperature cited by Jones, McKinney, and Fellows(*8*); but, as far as known, no controlled experiment has been carried out to determine this point. In view of this fact, it was decided to devise a simple, controlled experiment to determine the relation of variable and constant soil temperatures in connection with the Helminthosporium disease.

Obviously, when variable temperatures are worked with, an infinite number of combinations may be employed. In this experiment it seemed wise to employ the simplest combination possible which would enable a comparison to be made between the disease-producing influence of controlled variable soil temperatures and the influence of a constant soil temperature equivalent to the mean of the variable. It was decided, therefore, to alternate the soil temperature as uniformly as possible between 14° and 30° C. once every 12 hours; that is, the soil was to reach the maximum of 30° during the afternoon (between 1 and 2 o'clock) and to reach the minimum of 14°, 12 hours later (between 1 and 2 a. m.).

These temperatures were selected because they represent a reasonable soil fluctuation under field conditions, and because they lie on one side of the apex of the disease curve established by the "constant" soil temperature experiments with Harvest Queen wheat, as shown in figure 1. The particular time interval used was selected, not only on account of the fact that it conformed nearly to the condition in nature but because it divided the time between the upper and lower temperature range into equal intervals.

Three additional series were operated at constant temperatures of 14°, 22° (mean of 14° and 30° C.), and 30°, in conjunction with the alternating (14° to 30°) series.

The methods of conducting this experiment were the same as those used throughout the constant temperature series. One tank was devoted to each temperature and five pots were used in each tank, four of which contained the inoculated plants and one the uninoculated control plants. Harvest Queen wheat seed, Helminthosporium culture 392, and sterilized loam soil containing 33 per cent of moisture, water free basis, were used.

Soil temperatures in the constant series were controlled as described for the previous constant temperature experiments. In the case of the alternating temperature series control was obtained by means of a soil thermograph which was remodeled to serve both as a recording thermograph and a thermostat. By means of adjustable platinum points fixed to the inking arm and to the lever staff which is used to hold the inking arm away from the drum while changing records, it was possible to operate an electric spring switch and a water valve when the minimum or maximum temperatures were reached.

For this experiment an electric heater was obtained which raised the temperature of the water from 14° to 30° C. in approximately 12 hours. In addition, a flow of cold water was passed through a swivel valve which was so regulated that it delivered sufficient water to lower the tank water temperature from 30° to 14° in approximately 12 hours. The heater and valve were then operated by an electric current controlled by the adjustable platinum contacts, set at 14° and 30°, on the recording soil thermograph. The control apparatus required setting after each operation.

Owing to the slight irregularity in the water supply and to imperfections in the control apparatus there were some slight variations in the soil temperature curves shown in figure 2, but in the main these curves seem fairly satisfactory and should justify consideration of the disease data obtained therefrom.

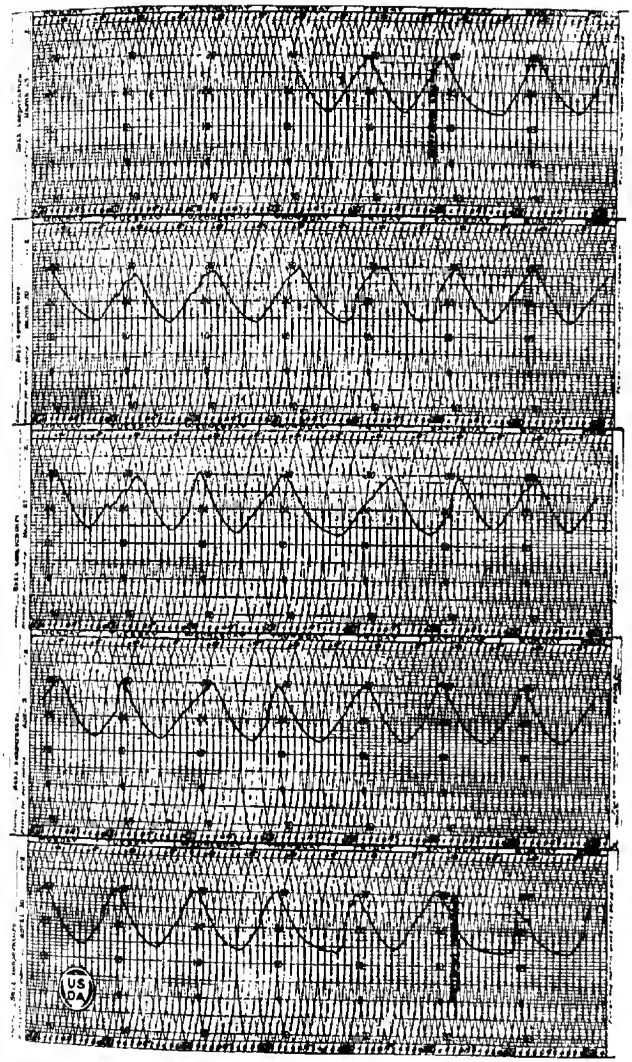


FIG. 2.—Soil thermograph records made during the experiment on alternating soil temperatures.

Results

Table V summarizes the results obtained in this experiment. From these data it will be seen that there was practically no difference between the amount of infection produced in the series beld at 22° C. and that wbicb was alternated between 14° and 30°. While there was a slight difference, it will be noted that the results of the seed inoculation series tend to neutralize those of the soil inoculation series; and in addition to this the variations are easily within the limits of experimental error.

Table V.—*Comparisons between the amounts of Helminthosporium infections on Harvest Queen wheat seedlings grown in soil held at constant temperatures of 14°, 22 and 30° C. and those on similar seedlings grown in soil at temperatures alternating daily between 14° and 30°*

Soil temperature.	Seed inoculation.		Soil inoculation.	
	Number of plants.	Infection rating.	Number of plants.	Infection rating.
°C.				
14 constant	108	9.4	109	11.[illegible]
22 constant	105	19.6	104	25.[illegible]
14 to 30 alternating	108	20.0	109	24.[illegible]
30 constant	86	75.0	84	85.[illegible]

While the results of this experiment show tbat plants grown at the mean temperature suffered practically the same degree of infection as those grown at the alternating temperatures, it should not be understood that this concept necessarily can be applied to all the possible combinations of time and temperature which might be arranged in experiments on this disease or on other diseases. Tbe results of the constant temperature experiments cited herein show that time is an important factor in disease development; and, undoubtedly, prolonged periods of favorable temperatures do tend to produce more disease than short periods of such temperatures.

Doubtless the relative position of the maximum and minimum temperatures selected on the disease curve established by the constant temperature experiment will influence results materially. It would seem probable that the results obtained in the alternating temperature experiment can hardly be expected to hold except when the maximum and minimum temperatures lie on the same side of the apex or optimum point of the disease curve established by constant temperature experiments. In view of the results of many physico-chemical experiments it does not seem reasonable to believe that the results above set forth would have been obtained if, for instance, the maximum and minimum temperatures bad been selected in such a way as to include between them the apex or optimum of the constant-temperature disease curve. Further study is planned in connection with the various phases of the problems thus suggested.

In his study on Fusarium blight, Dickson (4) reports that a short exposure to high temperatures during the germination period unbalanced the wbeat seedling and thus made it susceptible to the parasite. The writer has not noted this relation in connection with the Helminthospo-

rium disease, even in connection with the alternating soil temperature experiment cited above, but doubtless the relation of such high temperature to the previous and following temperatures to which the plant is submitted has some bearing on this point.

SOIL MOISTURE STUDIES

Three greenhouse experiments have been conducted in connection ith the soil-moisture studies. In the case of experiments 1 and 2 all f the plants were grown at the same greenhouse temperature (15° to 5° C.) during the experiments. In the case of experiment 3, the ioisture study was combined with the fifth soil-temperature experiment ith Harvest Queen wheat.

EXPERIMENTAL METHODS

The methods used in these experiments were the same as those employed ı the soil-temperature experiments. In all cases disinfected seed was ioculated with a water suspension of conidia just before sowing. Seeding as not done until the soil moistures had been carefully adjusted on a asis of the usual soil-moisture tests.

During the period of experiment the pots were weighed daily and ioisture adjustments made as needed. No difficulty was experienced ı adjusting the middle and higher moistures, but there was some diffiılty in adjusting the lower ones on account of uneven moisture disibution. This adjustment was facilitated, however, by applying water ten around the edge of the soil next to the pot wall and by keeping light dust mulch on the surface.

In experiments 1 and 2, metal pots 5 inches in diameter and 9.5 inches ep were used; in experiment 3, metal pots 8 inches in diameter and 5 inches deep were used.

ABLE VI.—*Results of experiments on the relation of soil moisture to the infection of Marquis and Harvest Queen wheat seedlings by Helminthosporium sativum when artificially inoculated seed was sown in a sandy loam soil having a moisture-holding capacity of 36 per cent, at Madison, Wis., in 1922*

Experiment 1.			Experiment 2.		
Marquis seed sown, culture 51a used. Experiment started Jan. 5, 1922; ended Jan. 30, 1922.			Harvest Queen seed sown, culture 392 used. Experiment started Feb. 28, 1922; ended April 12, 1922.		
Percentage of moisture-holding capacity.	Number of plants.	Infection rating.	Percentage of moisture-holding capacity.	Number of plants.	Infection rating.
22.2	0	0	27.7	80	0.8
33.3	168	22.6	33.3	80	13.0
44.4	173	29.1	44.4	80	18.3
55.5	163	50.4	55.5	75	26.5
66.6	151	64.3	66.6	69	30.5
77.7	126	48.2	77.7	13	31.2

TABLE VII.—*Results of an experiment with Harvest Queen wheat, combining a study of soil moisture and soil temperature (fifth series), in loam soil having a moisture-holding capacity of 67 per cent, using culture 392 on seed sown May 4, 1922, experiment ending May 26, 1922, at Madison, Wis.*

Experiment 3.

Soil temperature.	Soil moisture, on basis of moisture-holding capacity.							
	37.3 per cent.		46.2 per cent.		55.2 per cent.		62.6 per cent	
	Number of plants.	Infection rating.	Number of plants.	Infection rating.	Number of plants.	Infection rating.	Number of plants.	Infection rating.
°C.								
12	59	8.3	59	16.8	53	14.3	55	18.6
16	60	18.5	57	32.8	60	10.0	58	15.
20	53	32.3	60	53.7	55	66.6	55	45.
24	58	20.4	57	60.6	51	73.8	59	80.
28	60	35.4	53	74.8	58	82.7	56	86.
32	58	9.8	56	8.9	59	32.2	59	42.
34.5	54	8.0	58	7.3	53	15.0	45	3.

RESULTS

Tables VI and VII and figures 3 and 4 give the results of the soil moisture experiments. In general all of the data thus far obtained indicate that relatively high soil moistures favor the Helminthosporium disease of wheat. It is of interest to note the joint influence of soil temperature and moisture in experiment 3 as shown in Table VII and figures 4 and 5. In figure 5 it will be noted that the temperature optimum for disease development remained constant at all the soil moistures. Reference to figure 4, however, will show that the soil moisture optima were shifted when the soil temperature, as changed, the higher temperatures enabling

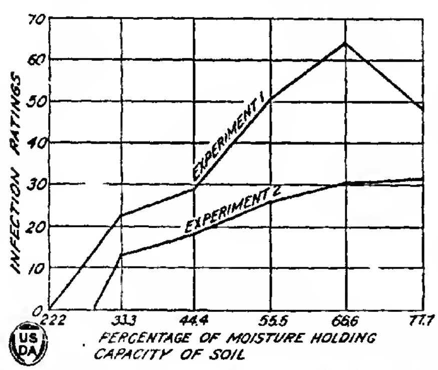


FIG. 3.—Graph showing the amounts of Helminthosporium infection on the subterranean parts of wheat seedlings grown at different soil moistures with other factors as uniform as possible, in experiments 1 and 2. Tabular results are given in Table VI.

the highest moistures to produce the maximum quantity of disease. The results indicate that the moisture optimum tends to drop in percentage as the soil temperature lowers. The irregularities in the low moisture curve in figure 5 and those in the 12° and 34.5° C. curves in figure 4 are not considered significant, since these curves represent the unfavorable extremes of the factors under study. Slight irregularities in other factors undoubtedly register themselves in a more pronounced manner when unfavorable soil moistures and temperatures are maintained, thus making it difficult to get the true expression of the influences of these two latter factors

Owing to the limited data available at this time, it is not possible to nalyze the results of experiment 3 with complete satisfaction. However, he present evidence seems to inlicate that soil temperature may e a more influential factor than oil moisture in connection with he development of the phases of e Helminthosporium disease nder discussion.

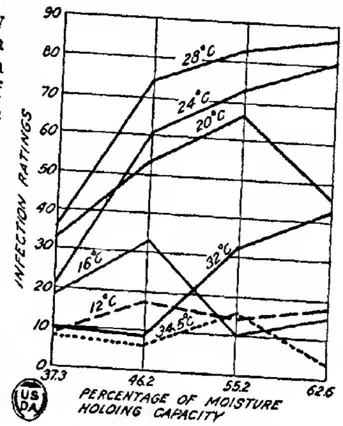
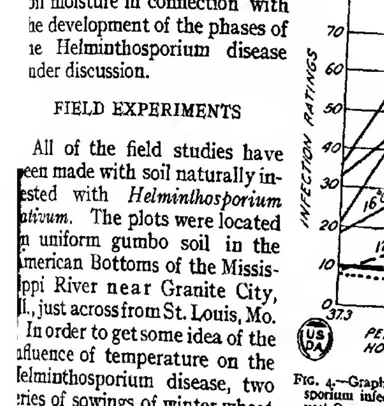


FIG. 4.—Graph showing the amount of Helminthosporium infection on the subterranean parts of Harvest Queen wheat seedlings grown at different soil moistures when the soil temperatures were varied simultaneously. Note the rather consistent influence of temperature on the shifting of the moisture optima. Tabular results are given in Table VII.

FIELD EXPERIMENTS

All of the field studies have een made with soil naturally inested with *Helminthosporium ativum*. The plots were located n uniform gumbo soil in the merican Bottoms of the Missisppi River near Granite City, l., just across from St. Louis, Mo.

In order to get some idea of the nfluence of temperature on the elminthosporium disease, two ries of sowings of winter wheat ere made at intervals during e autumns of 1920 and 1921. ach sowing consisted of a ot the width of an ordinary grain drill (54 inches) sown across the fested land. In 1920 these plots were 2 rods long and in 1921 they were 5 rods long. In 1920 Early May and Harvest Queen varieties were used and in 1921 Turkey and Harvest Queen were used. The dates of sowing are given in Tables VIII and IX.



5.—Graph showing the amount of Helminthosporium infection the subterranean parts of Harvest Queen wheat seedlings grown different soil temperatures and soil moistures. Same data as wn in figure 4, but plotted against soil temperatures instead soil moistures. Note that varying the soil moisture did not se the temperature optimum to shift in any case. Tabular lts are given in Table VII.

Owing to the distance of the field plots from the laboratory at Madison, Wis., and to difficulties in connection with getting some one to obtain accurate soil temperature and moisture records, it has been necessary to take the air temperature and precipitation data from the reports of the United States Weather Bureau at St. Louis, Mo. While se records do not represent the exact temperature and moisture ditions on the experimental plots, they approximate the general

trend of these factors very closely, and it is felt that they can be used safely as a basis for comparison.

Table VIII.—*Amount of autumnal infection by Helminthosporium on the underground parts of Early May and Harvest Queen wheats sown on different dates in a naturally infested field at Granite City, Ill., in 1920*

Variety.	Sowing dates.	Approximate mean temperature during growing period in fall.	Approximate total rainfall in inches during growing period in fall.	Fall data.		Spring data [a]	
				Date of observation.	Percentage of tiller infection.	Date of observation.	Percentage of tiller infection.
		°F.					
Early May....	Sept. 21	61.9	2.84	Nov. 12	64.7	May 13	91.05
Do........	Oct. 4	59.4	2.72	...do.....	26.3	...do.....	88.00
Do........	Oct. 11	57.6	2.72	...do.....	18.5	...do.....	74.24
Harvest Queen.	Sept. 21	61.9	2.84	...do.....	61.7	No data taken on Harvest Queen on account of complications from rosette disease.	
Do........	Oct. 4	59.4	2.72	...do.....	45.1		
Do........	Oct. 11	57.6	2.72	...do.....	10.9		

[a] These data are based on determinations which were very kindly made by Dr. R. W. Webb of the Office of Cereal Investigations.

Table IX.—*Amount of autumnal infection by Helminthosporium sativum in Turkey and Harvest Queen wheats sown on different dates in a naturally infested field at Granite City, Ill., in 1921*

Variety.	Date sown.	Date removed.	Approximate mean temperature during fall growing period.	Approximate mean rainfall during fall growing period.	Age of plants in days from seeding.	Percentage of plants infected.	Degree of infection.
			°F.	*Inches.*			
Turkey............	Sept. 20	Oct. 17	63.6	3.46	27	93.10	Abundant.
Do............	Oct. 1	Oct. 26	59.9	.70	25	18.20	Slight.
Do............	Oct. 12	Nov. 8	57.8	.97	27	11.10	Very slight.
Do............	Oct. 19	Nov. 17	54.0	1.23	29	13.40	Do.
Do............	Oct. 27	Nov. 21	49.8	4.91	25	19.20	Trace.
Do............	Nov. 11	Dec. 12	44.3	4.86	31	6.74	Do.
Harvest Queen.....	Sept. 20	Oct. 17	63.6	3.46	27	64.50	Abundant.
Do............	Oct. 1	Oct. 26	59.9	.70	25	19.70	Slight.
Do............	Oct. 12	Nov. 8	57.8	.97	27	5.30	Very slight.
Do............	Oct. 19	Nov. 17	54.0	1.23	29	11.70	Do.
Do............	Oct. 27	Nov. 21	49.8	4.91	25	14.50	Trace.
Do............	Nov. 11	Dec. 12	44.3	4.86	31	3.70	Do.

In all of the field experiments the amount of disease is expressed on the basis of the percentage of the number of plants infected on the underground parts, chiefly the sheaths, culms, and subcrown internodes. No account of the severity of the infection of individual plants was taken in arriving at this percentage.

In 1920 the autumnal data on all the plantings were taken on November . Percentages were based on all the plants growing in 5 linear feet of ill row in each plot. These 5 linear feet consisted of five 1-foot sections, ir of which were taken 1 foot from the ends of the two drill rows adja-it to the outside drill rows, and the fifth from the center row of each it.

Reference to Table VIII will show that early seeding tends to increase amount of Helminthosporium infection on the underground parts of ieat plants. These results are in line with those obtained in the con-illed soil temperature and soil moisture experiments, as the earlier field wings were submitted to higher temperatures and moistures than the er sowings.

It is of interest to note the results obtained the following spring on ese same sowings of Early May wheat. On May 13 counts were made in same manner as in the tumn, and while the per-itages of infection had in-ased considerably over se recorded in November is noted that the general ationship between the sow-s was the same as in the l—that is, the early sow-s still showed the greatest ounts of infection. This licates that the influence the date of fall sowing on disease may extend con-erably into the spring wing season. These data o indicate that the ounts of infection in the sowings tend to catch with those in the early wings as the season ad-ices.

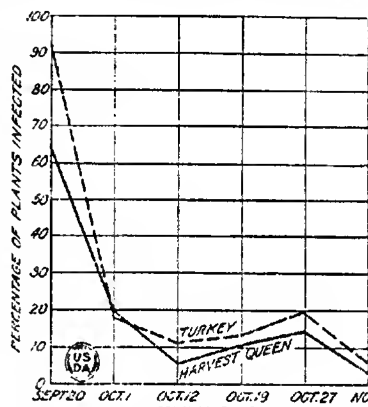


FIG. 6.—Graph showing the influence of date of seeding in autumn on Helminthosporium infection of the subterranean parts of Harvest Queen and Turkey wheat plants grown in the field. Tabular data are given in Table IX.

Spring data on the Helminthosporium disease were not taken on the rvest Queen plots, owing to complications from the rosette disease, ich attacks this variety but does not affect Early May wheat.

Undoubtedly the time factor played a considerable part in the results this experiment, but it seems rather doubtful if this wholly accounts the differences in the amount of disease in the different sowings. In er to eliminate the time element as far as possible from the field experi-nts, another method for taking data was adopted in the 1921 field eriments. Instead of making the disease determinations for all the ts at the same time, they were made as nearly as possible at a given e after the date of sowing of each plot. Three linear yards of plants e collected from each plot, 1 yard from near each end and 1 yard from center of the middle drill rows.

All data obtained in this experiment are shown in Table IX.

From these results and the curves shown in figure 6 it is evident that amount of disease tends to be greater when high temperatures and tively high moistures prevail.

It will be noted that the high rainfall during the growth periods of the later sowings in 1921 tended to raise the disease curves, but in the case of the last sowing it would seem that the decided drop in temperature had more influence on the disease than the marked rise in the soil moisture, since the disease curve went down at this time.

While it was not possible under the prevailing conditions to obtain the data on the several plots at exactly the same interval after seeding, this was done at intervals sufficiently close, as shown in Table IX, practically to eliminate the time element, except possibly in the case of the seedings made on October 19 and November 11. In these cases, however, the increased time period should have increased infection, but it did not seem to influence the results seriously.

In general, the results from the field experiments are in line with those obtained in the constant temperature experiments conducted in the greenhouse. This seems to strengthen the idea that the results of the latter experiments are a safe index to the soil temperature influence on the phases of the Helminthosporium disease under consideration.

DISCUSSION

While the foregoing results are considered as preliminary in nature, it seems evident that soil temperature and soil moisture are important factors in connection with the development of the Helminthosporium disease on the subterranean parts of spring and winter wheat and spring barley. Whether or not these are the most important environmental factors can not be determined from the data at hand. In this connection it is of especial interest to note that Hungerford (5) has observed severe Helminthosporium injury to wheat plants in Idaho only in the dry-land regions; and it is of further interest to note that he considers the trouble to be favored by a cold, wet spring followed by hot, dry weather. Obviously, these observations involved many variable factors the relative importance of which is not known at this time.

Other factors than temperature and moisture undoubtedly influence the development of the Helminthosporium disease. This conclusion is supported by the fact that there has been some shifting in the temperature optima of the several controlled experiments presented in this work. Dickson (4) has noted that light exerted an influence on his soil temperature experiments with the Fusarium blight of wheat, and it may be that there was such an influence on the writer's results with the Helminthosporium disease. As yet, however, too little evidence is at hand to warrant a direct statement on this point.

By way of comparison it is of interest to note the differences in response between the Fusarium and Helminthosporium seedling diseases on wheat. Results obtained by Dickson (4) in his study of Fusarium blight show that Turkey wheat (winter) is attacked, on the average, more vigorously at 28° C., whereas the writer's results with the Helminthosporium disease show that Harvest Queen (winter) wheat is attacked, on the average, more severely at 32°. In the case of Marquis wheat the results are the more striking in that Dickson's average data show a bimodal curve with the optimum at 20°, whereas the same variety shows a much higher temperature optimum (28°) for the Helminthosporium disease with no indication of bimodal tendency in the average data. A few of the writer's experiments with the Helminthosporium disease have shown a very

light bimodal tendency, but this phenomenon has been discounted on he basis of experimental error and because the temperature optimum for he Helminthosporium disease probably is not a decidedly critical point, ut a rather limited range..

As Dickson gives only averages of a number of experiments, it can not e determined whether he is dealing with an actual or an apparent bimodal ondition in Marquis wheat. It would seem that the interpretation of a ouble apex in a curve which represents the average results of a number f individual experiments must be considered from at least two angles: 1) As the possible expression of shifting optima in the several experiments making up the average, and (2) as the expression of a true bimodal eaction. In the second case we would, and in the first case we would not, xpect to find the bimodal character showing up in the individual experiments. Therefore, an analysis of the data from the individual experiments would seem necessary to interpret any bimodal tendencies. It ould seem, therefore, that Dickson's average data may represent only shifting optimum.

While the data herein presented indicate that the date of seeding inuences the severity of the Helminthosporium disease in winter wheat, ositive recommendations concerning a general seeding practice can not e offered until field sowings have been made with spring wheat and bary, and until more work has been done on the susceptibility of the plants nder different conditions and at different stages in their development. his seems especially true when it is considered that spring wheat and arley develop during a period of rising temperatures, whereas winter heat is first subjected to a period of descending temperatures, then to w temperatures fairly continuously, and later to rising temperatures. bviously, it is not safe to apply the results of field experiments with inter wheat to spring wheat or barley by recommending early planting the latter two cereals, but it does seem safe to assume that the late lanting of winter wheat, when other more important factors are not fected adversely, will tend to reduce the amount of Helminthosporium jury to the underground parts. Proper soil drainage also should aid reducing the disease.

SUMMARY

(1) *Helminthosporium sativum* P. K. and B. is a vigorous parasite, nder certain conditions, on all parts of wheat and barley plants.

(2) *H. sativum* has been claimed by certain workers to be the direct use of the rosette disease of wheat (sometimes called footrot and ke-all), but as yet there is no positive proof of this causal relation.

(3) In certain districts, especially in the spring wheat belt, the Helminosporium disease is at times very severe.

(4) Controlled greenhouse experiments and field experiments were ade to study the influence of soil temperatures and soil moistures the infection of the subterranean parts of winter and spring wheat d barley plants.

(5) In these studies fourteen constant soil temperature experiments d one controlled, alternating soil temperature experiment were concted in the Wisconsin soil-temperature tanks. Three soil-moisture periments were made in the greenhouse, one of which was conducted conjunction with a soil-temperature series.

(6) Two field experiments were conducted in naturally infested soil located in the American Bottoms of the Mississippi River near Granite City, Ill., opposite St. Louis, Mo.

(7) The results of all the experiments show that the Helminthosporium disease as it occurs on the underground parts of wheat and barley is influenced by soil temperature and soil moisture.

(8) The disease developed at all temperatures used between the extremes of 8° and 35° C., but infection was greatly reduced toward the extremes.

(9) The optimum soil temperature for the disease on Marquis (spring) wheat and on Hanna and Hannchen (spring) barleys was found to be 28° C. For Harvest Queen (winter) wheat the optimum was 32° C.

(10) There was some shifting in the optima of the several experiments, but it was limited to the high temperatures. This shifting is explained on a basis of other factors than moisture which were not uniformly controlled throughout all of the experiments. A control of such uncertain factors will make possible a more accurate determination of the temperature optima in future experiments.

(11) The disease seems to attack barley more freely than wheat at temperatures below 16° C.

(12) In all experiments Marquis wheat has shown the highest susceptibility to the disease.

(13) An experiment was conducted to determine the influence of controlled alternating soil temperatures on the disease in comparison with a constant temperature equivalent to the mean of the alternating series.

(14) Essentially the same amount of disease developed at the soil temperatures which alternated between 14° and 30° C. every 12 hours as developed at the constant mean temperature of 22°.

(15) These results are preliminary and represent but one simple combination of time and temperatures, and, therefore, should not be given too wide an application. However, they do indicate that the constant temperature method probably gives a fair index to the influence of soil temperatures under field conditions.

(16) Two soil-moisture experiments conducted in the greenhouse show that high soil moistures favor the disease. A third moisture experiment combined with a soil-temperature series also shows that high soil moisture is more favorable to the disease at temperatures of 24° C. and above.

(17) The results of this combined soil moisture and temperature experiment indicate that the temperature optimum is not altered by changes in soil moisture, whereas changes in soil temperature do seem to cause rather regular shifting in the soil moisture optimum. The temperatures at and above 24° C. favor a high moisture optimum, while temperatures below 24° C. seem to favor low moisture optima.

(18) Two field experiments show that there is a direct correlation between soil temperature and soil moisture and the development of the disease. Early-sown winter wheat is more severely affected by the disease than late-sown winter wheat. These results are in direct line with the controlled experiments conducted in the soil temperature tanks since early sowings are subjected to higher soil temperatures than are the late sowings.

LITERATURE CITED

(1) BECKWITH, T. D.
1910. MYCOLOGICAL STUDIES UPON WHEAT AND WHEAT SOILS TO DETERMINE POSSIBLE CAUSES IN DETERIORATION IN YIELD. *In* Science, n. s., v. 31, p. 798.

(2) BOLLEY, H. L.
1910. CONSERVATION OF THE PURITY OF SOILS IN CEREAL CROPPING. *In* Science, n. s., v. 32, p. 529–541.

(3) ———
1913. WHEAT. N. Dak. Agr. Exp. Sta. Bul. 107, 94 p., 45 fig.

(4) DICKSON, James G.
1923. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON THE DEVELOPMENT OF THE SEEDLING-BLIGHT OF WHEAT AND CORN CAUSED BY GIBBERELLA SAUBINETII. *In* Jour. Agr. Research, v. 23, p. 837–870, 15 fig., 6 pl. Literature cited, p. 869–870.

(5) HUNGERFORD, C. W.
1922. [NOTES ON THE HELMINTHOSPORIUM DISEASE IN IDAHO DURING 1921.] *In* U. S. Dept. Agr. Bur. Plant Indus., Plant Disease Survey Bul. Sup. 21, p. 196.

(6) JOHNSON, Edward C.
1914. A STUDY OF SOME IMPERFECT FUNGI ISOLATED FROM WHEAT, OAT, AND BARLEY PLANTS. *In* Jour. Agr. Research, v. 1, p. 475–490, pl. 42–43. Literature cited, p. 487–489.

(7) JONES, L. R.
1921. EXPERIMENTAL WORK ON THE RELATION OF SOIL TEMPERATURE TO DISEASE IN PLANTS. *In* Trans. Wis. Acad. Sci., Arts and Letters, v. 20, p. 433–459, pl. 33–37.

(8) ——— MCKINNEY, H. H., and FELLOWS, H.
1922. THE INFLUENCE OF SOIL TEMPERATURE ON POTATO SCAB. Wis. Agr. Exp. Sta. Research Bul. 53, 35 p., 9 fig., 5 pl. Literature cited, p. 34–35.

(9) MCKINNEY, Harold H.
1921. THE SO-CALLED TAKE-ALL DISEASE OF WHEAT IN ILLINOIS AND INDIANA. (Abstract.) *In* Phytopathology, v. 11, p. 37.

(10) ———
1922. THE HELMINTHOSPORIUM DISEASE OF WHEAT AND THE INFLUENCE OF SOIL TEMPERATURE ON SEEDLING INFECTION. (Abstract.) *In* Phytopathology, v. 12, p. 28.

(11) ———
1923. INVESTIGATION ON THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL. *In* Jour. Agr. Research, v. 23, p. 771–800, 2 fig., 8 pl. Literature cited, p. 799–800.

(12) STAKMAN, Louise J.
1920. A HELMINTHOSPORIUM DISEASE OF WHEAT AND RYE. Minn. Agr. Exp. Sta. Bul. 191, 18 p., 5 pl.

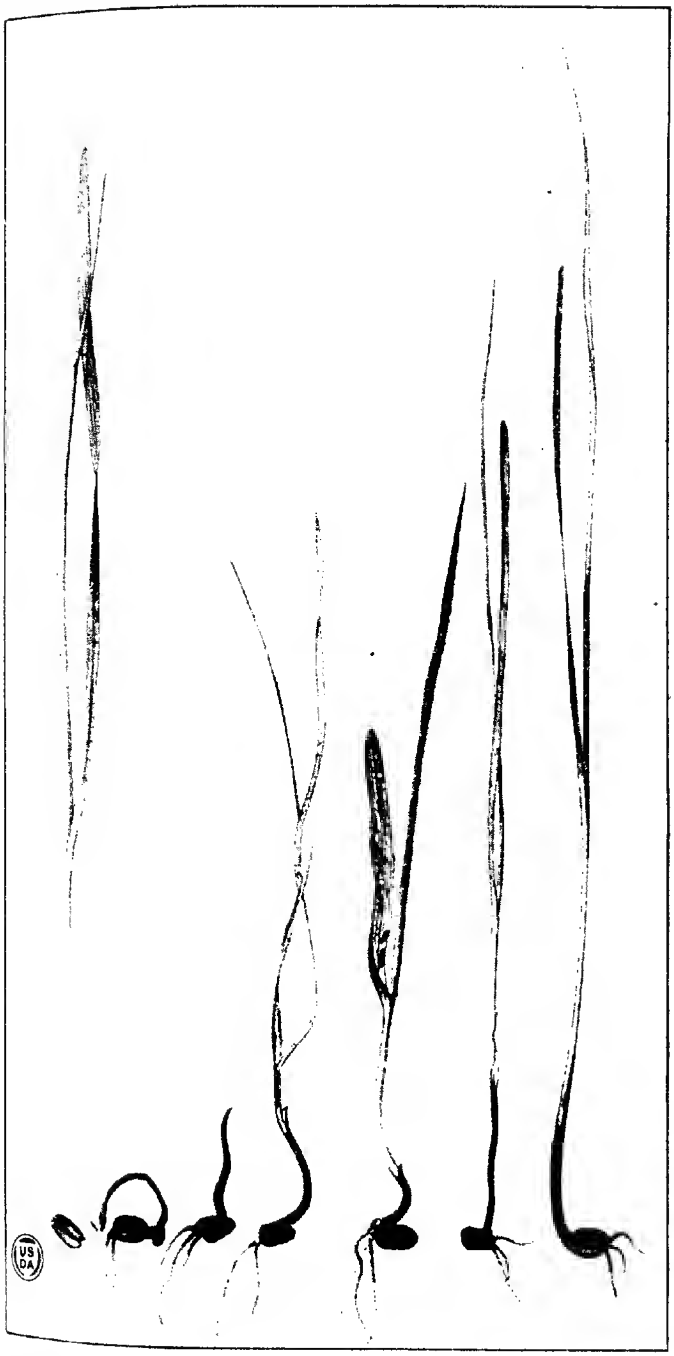
(13) STEVENS, F. L.
1919. FOOT-ROT DISEASE OF WHEAT—HISTORICAL AND BIBLIOGRAPHIC. *In* Ill. Nat. Hist. Surv. Bul., v. 13, art. 9, p. 259–286, illus.

(14) ———
1920. FOOT-ROT OF WHEAT. *In* Science, n. s., v. 51, p. 517–518.

(15) ———
1922. THE HELMINTHOSPORIUM FOOT-ROT OF WHEAT, WITH OBSERVATIONS ON THE MORPHOLOGY OF HELMINTHOSPORIUM AND ON THE OCCURRENCE OF SALTATION IN THE GENUS. *In* Ill. Nat. Hist. Surv. Bul., v. 14, art. 5, p. 77–185, illus. Literature cited, p. 171–178.

PLATE 1

Marquis wheat seedlings, healthy and artificially infected with *Helminthosporium sativum*. The healthy seedling at left was grown from disinfected seed sown in sterilized soil. The other six are of the same age and were grown from the same lot of disinfected seed but were sown in sterilized soil inoculated at sowing time with conidia of *H. sativum* grown in artificial culture (culture 51a). They show various types of primary infection.




A
B

PLATE 2

Marquis wheat seedlings, healthy and artificially infected with *Helminthosporium sativum*.

A.—Healthy plants from 115 disinfected kernels sown in steam-sterilized, uninoculated soil.

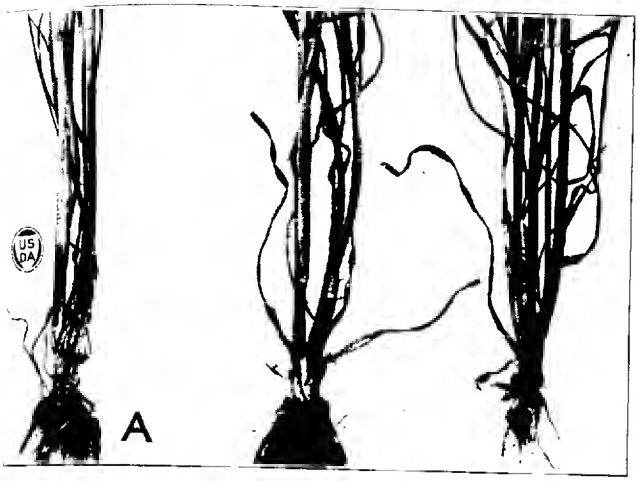
B.—Infected plants, same age as A, from 115 disinfected kernels sown in part of the same lot of soil inoculated at sowing time with a water suspension of conidia of *H. sativum* grown in pure culture (culture 51a) isolated from wheat.

PLATE 3

Basal portions of Early May wheat plants infected with *Helminthosporium sativum*

A.—Discoloration of bases of nearly mature plants grown in Helminthosporium infested soil in the field, characteristic of attacks of *H. sativum*.

B.—Discolored lesions on the bases of culms shown in A, the leaf sheaths having been removed. These are typical of basal discolorations caused by *H. sativum*.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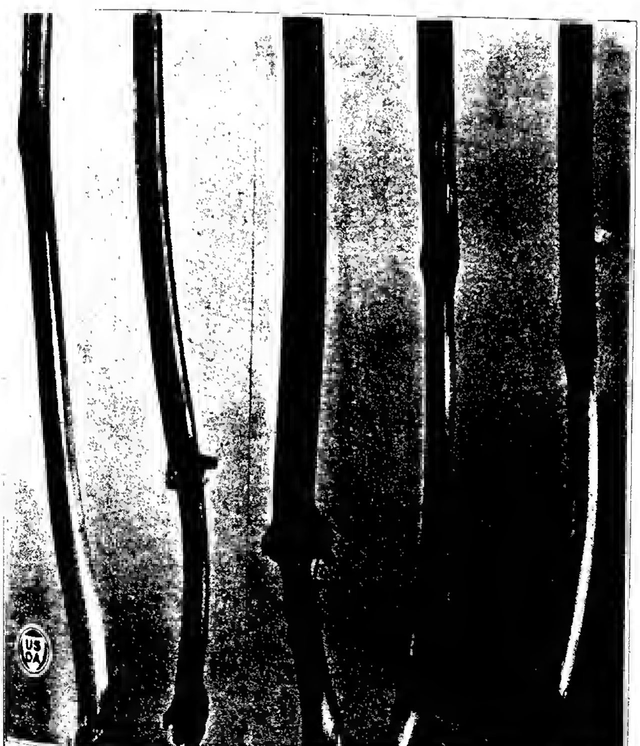

PLATE 4

Portions of Harvest Queen wheat leaves infected with *Helminthosporium sativum*. These leaf lesions with killed, bleached centers and dark brown margins are typical of secondary infections by *H. sativum*. × 2.

;IVE MOLDS AND THEIR PENETRATION INTO WOOD [1]

By Eloise Gerry [2]

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INTRODUCTION

During the war the necessity for shipping, kiln-drying, and otherwise iandling green wood which was destined for special and exacting uses, uch as the construction of airplane parts or artillery equipment, caused pecial consideration to be given to the possible effects of molds developng upon such material. Car loads of green lumber, for instance, often nolded heavily in transit; again, thick mats of mold developed at the eginning of dry-kiln runs under the favorable conditions offered by the elatively low temperatures and the high humidities used. Hence the uestion was repeatedly in the minds of inspectors and others responsible for the use of the wood, as to whether the molds produced more lamage than the frequently obvious superficial discoloration.

The following study was made at the Forest Products Laboratory in rder to determine, as far as it was possible to do so by the use of laboraory cultures and with the aid of the microscope, the extent of the peneration of common molds into such woods. This work was begun in June, 918, with some preliminary examinations of the effects of molds on vood. These showed no significant penetration of the cell walls. The esults here presented were obtained from a set of pure cultures prepared n June, 1919. These grew from that date until May, 1921, when they egan to show signs of drying out (Pl. 1, D. E. F, and I).

SPECIES OF WOOD

Four species of wood were selected for this study: Sitka spruce (*Picea itchensis* (Bong.) Trautv. and Mayer), a wood much used in airplanes, ow oak (*Quercus michauxii* Nutt.), a white oak, and a commercial red ak (*Quercus* sp.) used for propellers and for artillery wheels, and aspen *Populus tremuloides* Michx.), selected because it is a wood easily attacked y fungi.

Test blocks of these woods were cut and planed to a size of ½ × ½ × 1½ inches. All of the material was sapwood, with the exception of a ittle heartwood on the edge of some of the spruce blocks. Samples of ll the species were examined microscopically and found to be free from ungus hyphae before the blocks were prepared. Since the material

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[1] Accepted for publication July 10, 1923. A microscopic study of *Penicillium divaricatum*, *Monilia sitohila*, *Aspergillus niger*, *Ceratostomella* sp. and an unidentified form (No. 71218-1), grown for two years in ask cultures on blocks of aspen, Sitka spruce, white oak, and red oak respectively.

[2] Considerable assistance and many helpful suggestions were given in the course of the investigation by he members of the Laboratory of Forest Pathology of the Bureau of Plant Industry at Madison, Wis. The writer wishes particularly to acknowledge the help given in obtaining the cultures used, by Dr. C. J. Iumphrey, Dr. C. Audrey Richards, and Mrs. R. Lynnwalter. Helpful criticism and information on he use of special stains were also given by Dr. R. H. Colley and Dr. E. E. Hubert. In determining the xtent of the penetration of the molds, at the end of the test, some experiments in differential staining to acilitate observation and photomicrographing were made. In this work the suggestions and the advice of Dr. M. E. Diemer, Chemist in Forest Products, were of great assistance. For this help, as well as for that iven in making the photographs of the cultures and the photomicrographs, the writer wishes to express rateful appreciation to Dr. Diemer.

was air-dry, the blocks were placed in boiling distilled water for about five minutes and then in cold distilled water, in order to increase the moisture content. The blocks in bundles of five were then sterilized for an hour under 5 pounds pressure in an autoclave. Besides the test blocks, irregular-shaped culture blocks of mixed hardwoods were prepared to serve as a foundation in the flasks. These were kept in distilled water at about the boiling point for several hours. Cold water was added to saturate the blocks, and they were sterilized under 10 pounds pressure for an hour.

FLASK CULTURES AND THEIR INOCULATION

Twenty-two flask cultures (duplicate series for the eleven molds used) were then prepared as follows: A layer of cotton linters was placed on the bottom of a liter flask. One hundred and fifty cc. of distilled water were poured in and the cotton made to lie flat on the bottom of the flask. A number of culture blocks sufficient to cover the bottom were then added and on top of these were placed five test blocks of each species. The mouths of the flasks were closed with cotton plugs, capped with a layer of cotton and a layer of cloth and firmly fastened down.

The flasks were then sterilized, first for 30 minutes under 12 pounds pressure, then after 24 and after 48 hours, for 1 hour without pressure. In the meantime, water blanks were prepared and sterilized (20 cc. distilled water in a plugged test tube). Inoculations were made by the spore suspension method. A sterilized wire loop was dipped into the water blank and then inserted under sterile conditions in a stock culture of the mold to be used. The adhering spores were then deposited in the water in the test tube, which was shaken well and poured into the prepared flask.

SPECIES OF MOLDS

Penicillium luteum Zukal., *P. rugulosum* Thom, *P. divaricatum* Thom, *Aspergillus flavus* var., *A. niger* van Tiegh., *Monilia sitophila* (Mont.) Sacc., *Cephalothecium roseum* Cda., *Graphium* sp., *Ceratostomella* sp., *Mucor* sp., and an unidentified form which is very commonly found on Sitka spruce and red oak, were used.

The cultures showed growth three or four days after they had been inoculated. The early growth was abundant on the surface and fluffy (Pl. 1, A, B, C, G, H). The cultures were placed in a partially darkened cabinet, where they were frequently inspected, and here they were allowed to develop at room temperature for a period of almost two years. By May, 1921, signs of drying were apparent in the cultures, the general appearance of which at that time is indicated by Figures D, E, F, and I in Plate 1.

Inspection on this date showed one series of 11 cultures, one of each mold used, to be still somewhat moist and apparently alive. These were set aside in order that they might continue to grow and reach the greatest development possible.

Some of the duplicate cultures of these molds had become contaminated during the two years' growth, but five were pure—namely, *Aspergillus niger*, *Ceratostomella* sp., *Monilia sitophila*, *Penicillium divaricatum*, and the unidentified form (No. 71218–1). These were opened and transfers were made. The test blocks were then preserved for sectioning in a solution of formalin and alcohol (6 cc. 40 per cent commercial formalin to 100 cc. 50 per cent alcohol). The transfers were made under sterile

nditions. Slivers of wood from the interior of the test blocks, the rfaces of which had been washed off with a solution of mercuric chlorid, ere introduced into tubes of malt agar. These transfers indicated, after owing for a time, that each of the five original molds was alive and pure ell below the surface of the block.

The eleven cultures, which had been set aside for further growth, when spected on August 23, 1921, were apparently uncontaminated and ill growing. They were not reinspected until September 19, 1921, at hich time it was found that, after growing without apparent contami- ation for two years, the entire set had suddenly become seriously con- minated. It was concluded that there had probably been an infesta- on with mites. It was felt, however, that since the contaminating owth was of comparatively recent origin, information of some value ight be obtained by examining this material, although the results uld only be considered as supplementary and indicative, rather than nclusive. The examinations were made and yielded evidence in agree- ent with that obtained from the thorough study of the five pure cul- res of the first series, which finally were the source of all the pure lture material available for study as a result of this test.

METHODS OF EXAMINATION

Microtome sections were cut from the test blocks which had been pre- rved in formalin and alcohol and later soaked in glycerin and alcohol. re was taken to obtain areas from the interior, as well as from the sur- ce, of the block in order that the character of the penetration of the fferent organisms might be thoroughly examined. Some cross sec- ons were cut, usually midway between the ends of the block. The ngitudinal sections both radial and tangential were, however, on the hole, more satisfactory for study.

METHODS OF STAINING

The hyphae of these molds were for the most part colorless; often they ere very fine. Therefore, in order to facilitate the examination and e determination of the extent and character of the penetration of the olds by differentiating more clearly the mycelium from the host tissue, me experiments were made with stains. A number of stains used for is purpose have been described [3] (*5, 6, 8, 18, 19, 20*); these were tried the writer but did not appear entirely satisfactory. One very help- l staining method has been published since these tests were made (*11*). It was felt that since fungi are understood to contain a very distinctive embrane substance (chitin), some selective reaction could be found to ing out a contrast between the membranes of the fungous hyphae and the wood. At the suggestion of Dr. M. E. Diemer, experiments were ade with the application of gold and silver solutions. A preliminary te on the use of these solutions has been published (*9*). Some of the sults obtainable are illustrated in Plates 2, 3, and 4.

The methods employed with various reagents and the results obtained e given below in detail. The staining considerably facilitated the ob- rvations on the extent of the penetration of the molds in the case in nd, although insufficient time was spent to perfect, in a comprehensive anner, the technique of applying the methods developed. The stains ed were found to give good results in photomicrographing the material.

[3] Reference is made by number (italic) to "Literature cited," p. 228–229.

"BERLIN BLUE" REACTION

The reaction described is cited under tests for the localization o proteins by Dr. Sophia H. Eckerson[1] in "Notes on Microchemistry." It was applied with varying success. One excellent result is shown i Plate 2, A. In this instance the hyphae of the molds assumed a bright clear, blue color which caused them to stand out in striking contrast t the entirely uncolored background of the wood cells. The method use consisted in placing the sections in a dilute solution of potassium ferro cyanide (1 part potassium ferrocyanide to 20 parts water and 10 aceti acid, sp. gr. 1.063). After about an hour the sections were carefull washed with 60 per cent alcohol and a few drops of dilute ferric chlori were added. The hyphae immediately turned a clear transparent blue

SILVER SOLUTIONS

A saturated solution of silver nitrate in distilled water was prepared as a stock solution and used in varying dilutions. This solution was effective in practically all cases. The mycelium in sections soaked in silver nitrate for periods varying from one to two hours to as many days assumed an orange, dark brown, or, in one case, violet brown color in contrast to the constantly lighter color of the wood tissue.

Plate 2, D, shows what a striking differentiation may be obtained. In this specimen the organism was not a mold but a wood-destroying fungus. Plate 3, A, shows the mycelium of the mold *Monilia sitophila* in a piece of white oak. Here one of the worst difficulties encountered with this stain is apparent, namely, the precipitate which, although it does not interfere notably with the detection of the fungus, makes a dirty-looking preparation. No satisfactory means of removing the precipitate was devised. It occurred even with extremely dilute solutions. Whenever the mycelium was well stained, a precipitate might be found, although it was not necessarily present, as indicated by Plate 2, D. Dissolving the precipitate invariably also bleached the mycelium. Slight assistance was obtained by washing with ammonia, sometimes followed by very dilute acetic acid. Sets of sections were also suspended in the silver nitrate solution vertically on platinum hooks and kept in the dark and in the light, respectively. Although this tended to eliminate the precipitate, the resulting differentiation was not so marked as when the sections were laid flat in an ordinary staining dish. Treating the sections with glycerin tended to improve the quality of the differentiation secured. Long soaking (over-night) in dilute stain gave, on the average, good results. Permanent mounts of this material were made by passing the sections through the usual dehydrating alcohols, clearing in xylol, and mounting in Canada balsam. Crystals present in the wood often appear dark with this treatment.

Silver lactate was suggested for use instead of silver nitrate, and some was obtained through the courtesy of Dr. Alfred Koehler, of the University of Wisconsin. It was not found to be as effective as the nitrate, however. The precipitate was just as abundant and the mycelium was less well stained. It was particularly noticeable with this solution that in spruce the middle lamella and the "bars of Sanio" stained a marked orange, similar to the color acquired by the mold hyphae.

[1] ECKERSON, Sophia H. NOTES ON MICROCHEMISTRY. (Unpublished.)

GOLD SOLUTIONS

Of all the solutions used, c. p. gold chlorid in distilled water gave the nost satisfactory results. The best differentiation was obtained with ery dilute solutions (1 part gold chlorid to 2000 parts distilled water) in which the sections were allowed to stand for a considerable period, 24 ours or more (Pl. 2, B and C; Pl. 3, B; and Pl. 4). Greater contrast nd quicker response were obtained in some cases by giving the sections preliminary treatment with borax (sodium biborate) or with a 2 to 10 er cent solution of sodium acid sulphite, or of sodium thiosulphate photographic hypo). With the gold solutions the mycelium appears in arious shades of purples and reds against a paler or more bluish backround. A very clear differentiation is given, even in the case of the ery fine mycelial threads. Interesting differentiations in the various lements of the wood itself are brought out by this treatment (Cf. Pl. 3, B).

OTHER SOLUTIONS

Other chlorids, including those of mercury, platinum, and palladium lso were tried, but were found to be decidedly less effective than gold.

SELENIUM DIOXID

Some selenium dioxid crystals were obtained through the courtesy of rofessor Victor Lenher, department of chemistry, University of Wisonsin. The wood was colored scarlet (especially if heated) by solutions f various concentrations, but no differentiation was obtained.

EXTENT OF ATTACK OF MOLDS ON WOOD SPECIMENS

ASPERGILLUS NIGER

The culture of *Aspergillus niger* grew vigorously. It developed its haracteristic black spores on the surface of the blocks, as is indicated in late 1, G. The individual test blocks, when removed from the culture ask, were found to be more or less discolored on the surface, chiefly by he dark, powdery spores of the mold. The ends especially, which were ot smooth like the sides, were much affected. The sides showed slight iscoloration, but the interior of the blocks, except for the growth in the ores or vessels, appeared to the naked eye about as clean as at the beginng of the test. The exterior of the oak blocks was more discolored han that of the spruce and aspen material.

The mycelium of this mold was found chiefly in the vessel cavities. he hyphae developed abundantly in these open, readily accessible tubes nd were chiefly confined to them, as is illustrated by Plate 2, C. Practilly no penetration through the thick cell walls was found. The hyphae ere abundant in spruce (which has no vessels), but their course in this ecies was chiefly longitudinal in the tracheid cavities; there was a inimum number of crossings from cell to cell, and these appeared to be iefly through the pits or thin areas in the cell walls. The diameters of e hyphae were larger near the surface of the wood than below. Little no injury to the wood was apparent in the material.

CERATOSTOMELLA sp.

The blocks inoculated with *Ceratostomella* sp. did not show the chareristic bluing usually associated with its presence in nature. Otherise, the development of the culture was normal. Some of the hyphae

observed were very fine, especially in the aspen blocks. They were hyalin in many cases before staining reagents were applied. The appearance of the blocks is shown in Plate 1, H and I. The presence of a surface darkening is to be seen in the case of certain blocks in I. This darkening was especially marked on the ends of the blocks and the surfaces showed discolored streaks. The growth within the blocks was less abundant in the case of this mold than with the other four species. It tended to be localized near the surface especially. The vessels contained the most mycelium, but hyphae were also present to some extent in the rays and fibers of aspen and white oak. In spruce the development was chiefly in the tracheids, and the hyphae extended longitudinally near the surface. No such marked effects on the wood were produced in this culture as in those described and figured by Hubert (*10, 12*) who observed cell walls that were bored through and also exhibited surface thinning in instances where hyphae developed along the wall in contact with it.

MOLD 71218–1 (AN UNIDENTIFIED FORM COMMON ON SITKA SPRUCE AND RED OAK)

In the cultures of the unidentified mold No. 71218–1 the blocks showed a considerable dark discoloration on the surface. The development in aspen and spruce was not so vigorous as that of the other molds. The growth was chiefly longitudinal in the open cavities of the vessels, tracheids (Pl. 2, A), and resin passages. Except near the surface, the traversing of cell walls appeared to be reduced to the lowest degree consistent with progress from cell to cell. Mycelium was found, however, in apsen fibers, in spruce rays and in the rays and vertical parenchyma of the white oak specimens.

MONILIA SITOPHILA

The aspen blocks which had been inoculated with *Monilia sitophila* appeared clean for the most part, only slight darkening, probably due chiefly to water stain, occurring near the edges. The other species of wood showed dark spots, and here and there slimy mats of mycelium adhered to the blocks. The growth of mycelium within the blocks was, however, abundant. Large twisted hyphae were present, especially at the center of the aspen block. In this case the growth of the mold was not confined to the vessels but was abundant in the fibers, rays, and vertical parenchyma. Many of the hyphae bored through the cell walls and traveled across the grain as well as longitudinally. This was noted particularly in the white oak specimens (Pl. 3, A). The spruce, on the other hand, appeared to be attacked chiefly near the surface (Pl. 2, B). In that region the hyphae were large and abundant and showed less boring action on the cell walls than this fungus exhibited in the case of the other species of wood.

PENICILLIUM DIVARICATUM

The external effect of *Penicillium divaricatum* varied considerably with the different species of wood. The aspen test specimens were fairly clean looking to the naked eye, except for some spots and end darkening. The spruce, although it showed only slight discoloration of the ends, seemed softer than the normal wood of the species when it was cut in preparing the sections for microscopic study. The red oak blocks showed considerable end discoloration or darkening, and the white oak specimens had this appearance in a still more marked degree. Mats of mycelium

adhered to the wood and here and there dark areas were found on the sides of the blocks.

The development of the mycelium within the blocks was especially marked and abundant in the case of this species of mold. The hyphae not only extended longitudinally, but frequently also bored transversely through even the thicker cell walls. In aspen and the oaks the mycelium of this fungus was found abundantly in the rays, fibers, and vertical parenchyma, as well as in the vessels (Pl. 4). In the red oak particularly very fine hyphae, as well as coarse, vigorous ones were observed. In spruce the most abundant growth was near the surface, where very fine hyphae were produced, but the hyphae penetrated also to the very center of the block, traversing both the sapwood and the heartwood, a small amount of which was present in the test blocks. The tendency of the hyphae of this mold to bore through thick cell walls, especially in the aspen blocks, is clearly illustrated in Plate 4. Their penetration through the end walls of vertical parenchyma cells is shown in Plate 4, B. The attack of *Penicillium divaricatum* upon the wood cell walls was the most effective of any observed in the study.

DISCUSSION AND CONCLUSIONS

The test blocks were frequently much discolored and stained by the surface growth or spores of the molds or by water stain; but they were not appreciably softened, except in the case of *Penicillium divaricatum* on spruce, where the wood appeared unusually soft when sectioned with the microtome.

The development of the mold mycelium in the test blocks as observed under the microscope was found to vary considerably. Some of the molds showed more penetration of the cell walls than others, although practically all were found well below the surface of the blocks. Moreover, growth in the vessel cavities alone, such as was found in the case of red oak with *Aspergillus* sp. and *Ceratostomella* sp., presumably indicated less damage to the wood than would be expected in those cases where the hyphae were present in the rays and fibers, as was the case especially with *Monilia sitophila* and *Penicillium divaricatum* and also with other molds in white oak and aspen.

It is apparent from the results here shown that *Monilia sitophila* and *Penicillium divaricatum* penetrated the cell walls of the wood to a greater extent than did the other molds. Observations on the behavior of *Ceratostomella* sp., a blue stain fungus, made by others (*10*, *12*) have given evidence that this mold can also penetrate the cell walls and cause their thinning to a greater extent than was observed in the present test, but it is nevertheless maintained by pathologists that this does not materially affect the strength of the wood for *ordinary* commercial purposes.

It is apparent from the foregoing that the mycelium of certain molds may actually penetrate wood to a notable extent, even traversing thick cell walls (Pl. 4, A, C, and D). In general, however, it was observed that the tendency was to follow the cell cavities, especially those of the vessels or tracheids near the surface and (Pl. 2, B, C) to pass from cell to cell through the thin areas offered by the pits.

The effect of such an infection upon the strength of the wood has not been determined; but, until they are proved not guilty, it would appear that molds should be guarded against as much as possible in the endeavor to advance the cause of general lumber sanitation, and especially should molding be prevented in the case of material for exacting uses.

TABLE I.—*Results from microscopic examination of test blocks*

Molds.	Notes on chief location and character of growth within the blocks of the mold mycelium.			
	Aspen, *Populus tremuloides.*	A red oak, *Quercus* sp.	A white oak (cow oak), *Quercus michauxii.*	Sitka spruce, *Picea sitchensis.*
Aspergillus niger, 5118–2.[a]	*Abundant*, especially at center of block. Chiefly in *vessels*.	*Abundant*. Chiefly in *vessels*.	*Abundant*. Chiefly in *vessels*.	*Abundant*. Extending longitudinally more than a tracheid length. Chiefly in *tracheids*.
Ceratostomella sp., 82418–6.	*Limited* (localized). Best near surface. Abundant in *vessels*, *fibers*, and *rays*. Mycelium very fine.	*Moderate*. Chiefly in *vessels*.	*Abundant*. Chiefly in *rays*, *vertical parenchyma*, and *small vessels*. Some in *tracheids*.	*Limited*, longitudinally near surface only. Chiefly in *tracheids*.
Monilia sitophila, 61818–2.	*Abundant*. Large, twisted mycelium, especially toward center of block. Chiefly in *vessels*; some in *fibers*, *rays*, and *vertical parenchyma*.	*Abundant*. Chiefly in *vessels*, also in *rays*, and *vertical parenchyma*.	*Very abundant*. In *vessels*, *rays*, and *vertical parenchyma*. Passing through walls across the grain as well as longitudinally.	*Abundant* penetration, especially near surface. Chiefly in *tracheids* extending longitudinally. Some in *rays*.
Penicillium divaricatum, 5118–5.	*Abundant* in all directions. In *vessels*, *rays*, and *fibers*.	*Abundant*. Chiefly in *vessels*; large threads. Some fine in *tracheids*, *rays*, and *vertical parenchyma*.	*Abundant*. Chiefly in *vessels*, *rays* and *vertical parenchyma*; some in *tracheids*.	*Abundant*. Often very fine mycelium especially near surface, but penetrating to the center of the block in both sapwood and heartwood. Chiefly in *tracheids*.
Unidentified Form, 71218–1.	*Moderate*, especially developed near surface. Chiefly in *vessels*, also locally in *fibers*.	No material.	*Abundant*. Chiefly in large and small *vessels*, *vertical parenchyma* and *rays*.	*Moderate*. Some very fine mycelium. In *tracheids*, *resin passages* and *rays*. Rarely found passing through walls except near surface.

[a] Culture number in Forest Pathology files, Forest Products Laboratory, U. S. Department of Agriculture.

That cytolytic enzyms are produced by fungi, including some of those :lassed as molds, has been pointed out by various investigators (*1, 2*), *417, 3, 4 p. 231, 7, 13, 14, 15, 16, 17, 21, p. 331*). Some report attacks ,n the middle lamella, others on the cell walls. There is little which bears lirectly on wood although Ward (*22*) concluded: "It certainly looks is if *Penicillium* may be a much more active organism in initiating and :arrying on the destruction of wood than has hitherto been supposed, ind that it is not merely a hanger-on or follower of more powerful wood-lestroying fungi. It is also doubtless very independent of antiseptics."

Finally, as has been pointed out, it is clear that certain molds may ictually bore through cell walls, or produce a surface thinning, presuma-)ly through the activities of cytolytic enzyms (with an effect which hough probably limited is similar to that of a wood-destroying fungus). Ioreover, conditions which foster the growth of molds will also permit ither fungi to develop and spread. Hence, moldiness of material is an ndication that it may have been subjected to more or less undesirable onditions. Lastly, molds (commonly *Penicillium divaricatum*) are fre-juently isolated from seriously decayed or rotted wood, indicating that he molds flourish in that environment.

With these facts in mind it is obvious that the prevention of the mold-ng of lumber is desirable. Although no method of perfectly controlling t is known, a number of helpful methods have been, or are being devel-iped, by experiment. The conditions favorable to the development of nolds in wood are abundant warmth and moisture. Free access of air ends to lower moisture content. Hence the open piling of the material rith good opportunity for circulation of air is of considerable assistance n preventing the development of molds. This may also be accom-ilished with varying degrees of success by treating the lumber with anti-eptic solutions. In some localities, and under ordinary conditions, a hot olution of 4 to 8 per cent sodium carbonate (soda ash) or 5 to 11 per cent odium bicarbonate (baking soda) may be used successfully as a dip for he stock as it comes from the saw. These are not perfect protectors inder severe conditions, but either will assist in keeping the stock clean. here are other chemical dips, such as mercuric chlorid (0.1 per cent olution) which, because of its poisonous character, is not desirable, or odium fluorid (3 per cent solution) which will generally prevent blue tain but has not been found so successful with molds in general. Kiln-rying is an effective method of preventing infection and of killing molds lready present in lumber. Sometimes molds may develop abundantly n the early stages of a kiln run. Their growth may be stopped, however, y steaming the stock for one hour at 170° to 180° F. This treatment, ince the air is saturated, does not too rapidly dry the lumber.

SUMMARY

Pure cultures of five so-called molds, after growing in flasks for two ears were found to have developed mycelium in the wood below the sur-ace of the ½ x ½ x 1½ inch test blocks of aspen, Sitka spruce, red oak, and hite oak. The mycelium was present in the center of the hardwood locks. The penetration was chiefly through the natural openings—that ;, vessel or tracheid cavities, in the case of *Aspergillus niger* and *Cera-tomella* sp.

Monilia sitophila and *Penicillium divaricatum* showed the greatest mount of development in the different wood elements and a marked ten-

dency to traverse cell walls. The unidentified mold No. 71218-1 was also found to have entered the wood fibers and parenchyma as well as the open vessels and resin passages.

Water solutions of gold chlorid and also of silver nitrate, but to a less satisfactory extent, were found to give good differential staining, contrasting the mycelium with the host tissue so as to facilitate microscopic observation.

The fact that certain molds may destroy cell-wall substance and that many produce a surface discoloration makes it desirable to prevent the occurrence of mold in material to be subjected to especially exacting uses.

LITERATURE CITED

(1) ARTHUR, J. C.
1897. THE MOVEMENT OF PROTOPLASM IN COENOCYTIC HYPHAE. *In* Ann. Bot. v. 11, p. 491-507, 6 fig. Bibliographical footnotes.

(2) BARY, Anton de.
1886. UEBER EINIGE SCLEROTINIEN UND SCLEROTIENKRANKHEITEN. *In* Bot. Ztg., Jahrg. 44, p. 409-426.

(3) BEHRENS, J.
1898. BEITRÄGE ZUR KENNTNIS DER OBSTFÄULNIS. *In* Centbl. Bakt., [etc.], Abt. 2, Bd. 4, p. 514-522, 547-553, 577-585, 635-644, 700-706, 739-746, 770-777. Bibliographical footnotes.

(4) BOURQUELOT, Émile.
1893. LES FERMENTS SOLUBLES DE L'ASPERGILLUS NIGER. *In* Bul. Soc. Mycol. France, t. 9, p. 230-238.

(5) CHURCHMAN, John W.
1921. SELECTIVE BACTERIOSTATIC ACTION OF GENTIAN VIOLET. (Title.) *In* Science, n. s., v. 54, p. 326.

(6) COLLEY, Reginald H.
1918. PARASITISM, MORPHOLOGY, AND CYTOLOGY OF CRONARTIUM RIBICOLA. *In* Jour. Agr. Research, v. 15, p. 619-660, 1 fig., pl. 48-59. Literature cited, p. 655-659.

(7) CZAPEK, F.
1899. ZUR BIOLOGIE DER HOLZBEWOHNENDEN PILZE. *In* Ber. Deut. Bot. Gesell., Bd. 17, p. 166-170. Bibliographical footnotes.

(8) DICKSON, B. T.
1920. THE DIFFERENTIAL STAINING OF PLANT PATHOGEN AND HOST. *In* Science, n. s., v. 52, p. 63-64.

(9) DIEMER, M. E., and GERRY, Eloise.
1921. STAINS FOR THE MYCELIUM OF MOLDS AND OTHER FUNGI. *In* Science, n. s., v. 54, p. 629-630.

(10) HUBERT, Ernest E.
1921. NOTES ON SAP STAIN FUNGI. *In* Phytopathology, v. 11, p. 214-224, 4 fig., pl. 7. Literature cited, p. 223-224.

(11) ———
1922. A STAINING METHOD FOR HYPHAE OF WOOD-INHABITING FUNGI. *In* Phytopathology, v. 12, p. 440-441.

(12) ———
1922. SOME WOOD STAINS AND THEIR CAUSES. *In* Hardwood Rec., v. 52, no. 11, p. 17-19, illus.

(13) JONES, L. R.
1910. THE BACTERIAL SOFT ROTS OF CERTAIN VEGETABLES. II. PECTINASE, THE CYTOLYTIC ENZYM PRODUCED BY BACILLUS CAROTOVORUS AND CERTAIN OTHER SOFT-ROT ORGANISMS. *In* Vt. Agr. Exp. Sta. Bul. 147, p. 283-360, 10 fig. Bibliography, p. 357-360.

(14) MCBETH, I. G., and SCALES, F. M.
1913. THE DESTRUCTION OF CELLULOSE BY BACTERIA AND FILAMENTOUS FUNGI. U. S. Dept. Agr. Bur. Plant Indus. Bul. 266, 52 p., 4 pl. Bibliography, p. 47-50.

(15) MIYOSHI, Manabu.
1894. UEBER CHEMOTROPISMUS DER PILZE. *In* Bot. Ztg. Jahrg. 25, p. 1-28, pl. 1. Bibliographical footnotes.

(16) MIYOSHI, Manabu.
1895. DIE DURCHBOHRUNG VON MEMBRANEN DURCH PILZFÄDEN. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 28, p. 269–289, 3 fig. Bibliographical footnotes.

(17) NEWCOMBE, Frederick C.
1899. CELLULOSE-ENZYMES. *In* Ann. Bot., v. 13, p. 49–81. Bibliographical footnotes.

(18) PIANESE, Giuseppe.
1896. BEITRAG ZUR HISTOLOGIE UND AETIOLOGIE DES CARCINOMS. Beitr. Path. Anat. u. Allg. Path., Supplementheft 1, 193 p., 8 col. pl. Litteraturverzeichnis, p. 46–51, 164–168.

(19) SINNOTT, E. W., and BAILEY, I. W.
1914. SOME TECHNICAL AIDS FOR THE ANATOMICAL STUDY OF DECAYING WOOD. (Abstract.) *In* Phytopathology, v. 4, p. 403.

(20) VAUGHAN, R. E.
1914. A METHOD FOR THE DIFFERENTIAL STAINING OF FUNGOUS AND HOST CELLS. *In* Ann. Mo. Bot. Gard., v. 1, p. 241–242.

(21) WARD, H. MARSHALL.
1888. SOME RECENT PUBLICATIONS BEARING ON THE QUESTION OF THE SOURCES OF NITROGEN IN PLANTS. *In* Ann. Bot., v. 1, p. 325–357.

(22) ———
1898. PENICILLIUM AS A WOOD-DESTROYING FUNGUS. *In* Ann. Bot. v. 12, p. 565–566. Bibliographical footnotes. Also in Brit. Mycol. Soc. Trans., v. 1, p. 51–52. 1896–97.

PLATE 1

Mold cultures inoculated June, 1919.

Photographs taken July, 1920, when cultures were moist:

A.—*Penicillium divaricatum*. (Cf. D.)

B.—*Penicillium rugulosum*. Shows characteristic vigorous growth at this stage.

C.—*Monilia sitophila*. (Cf. F.)

G.—*Aspergillus niger*.

H.—*Ceratostomella* sp. Blue stain. (Cf. I.)

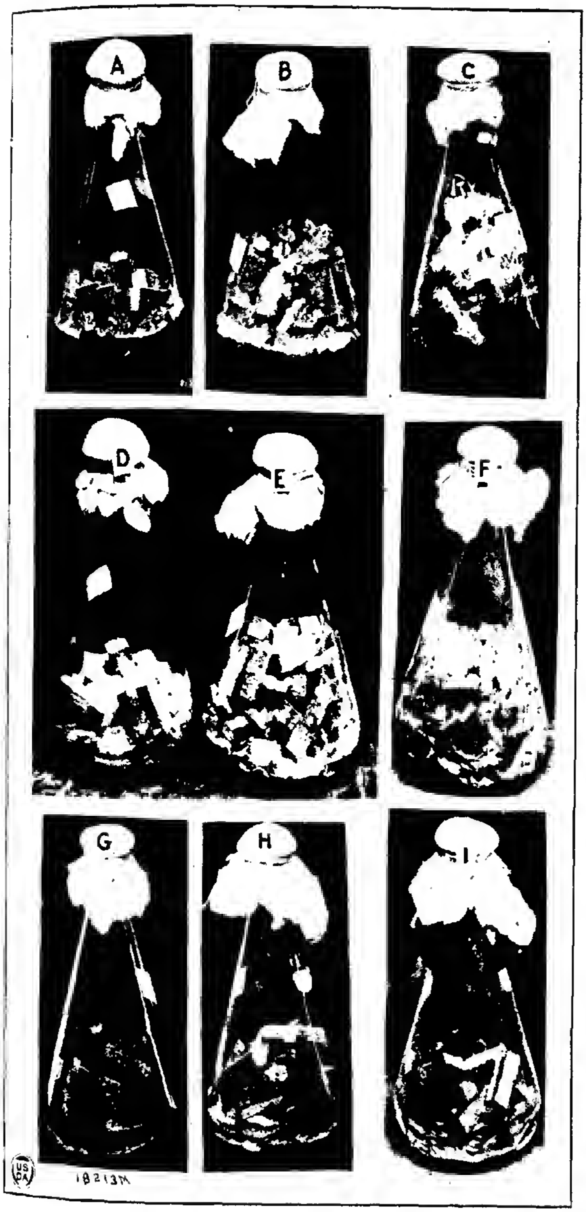
Photographs taken May, 1921, when cultures had considerably dried out:

D.—*Penicillium divaricatum*. (Cf. A.)

E.—*Penicillium rugulosum*. (Cf. B) Characteristic loss of fluffy appearance with time and drying out of culture.

F.—*Monilia sitophila*. (Cf. C.)

I.—*Ceratostomella* sp. Blue stain. (Cf. H.)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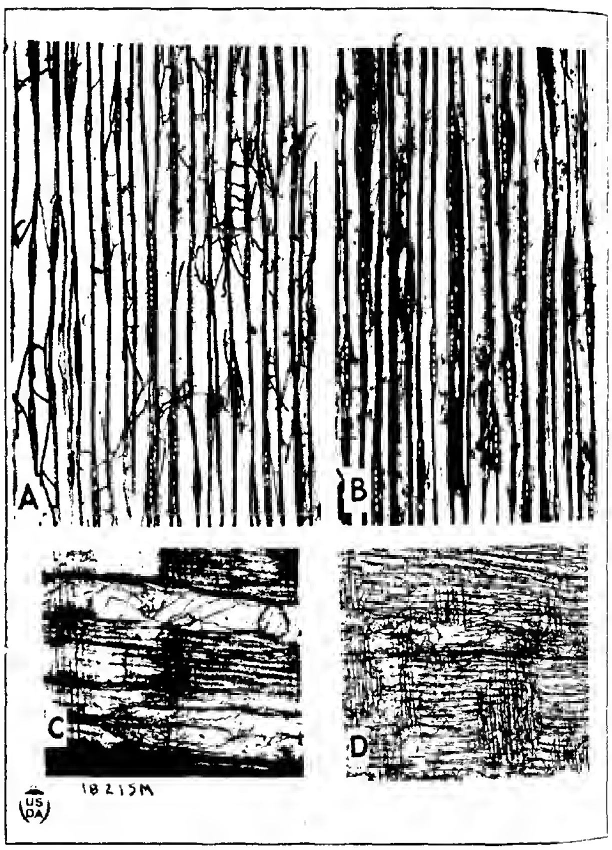
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PLATE 2

A.—Sitka spruce inoculated with an unidentified mold, commonly found on this species in nature. The mold mycelium, stained by the "Berlin blue" method, appeared as clear bright blue threads. Section cut near surface.

B.—Sitka spruce attacked by *Monilia sitophila*. Note development of large mycelia threads. Section stained with gold chlorid, applied after a treatment of 5 hours with sodium acid sulphite. Section was in the gold solution 20 hours.

C.—Aspen attacked by *Aspergillus niger*. Infection confined chiefly to the pores. This section was soaked 6 hours in borax (an unnecessarily long time), and left in gold chlorid solution 17½ hours.

D.—Maple attacked by a wood-destroying fungus. Dilute silver nitrate used very successfully as a stain. The wood appeared yellow and the fungus threads dark brown. There was no trace of precipitate in this case.

PLATE 3

A.—A white oak attacked by *Monilia sitophila*. Section stained with silver nitrate. The precipitate which is often troublesome with this stain is apparent here, yet the fungus is clearly differentiated.

B.—This unidentified fungus, present in some brash Sitka spruce from another investigation, is inserted because it illustrates the excellent differentiation, in the case of both wood and fungus, that was obtained with an overnight staining in a dilute solution of gold chlorid applied after a treatment of less than one hour with sodium acid sulphite.


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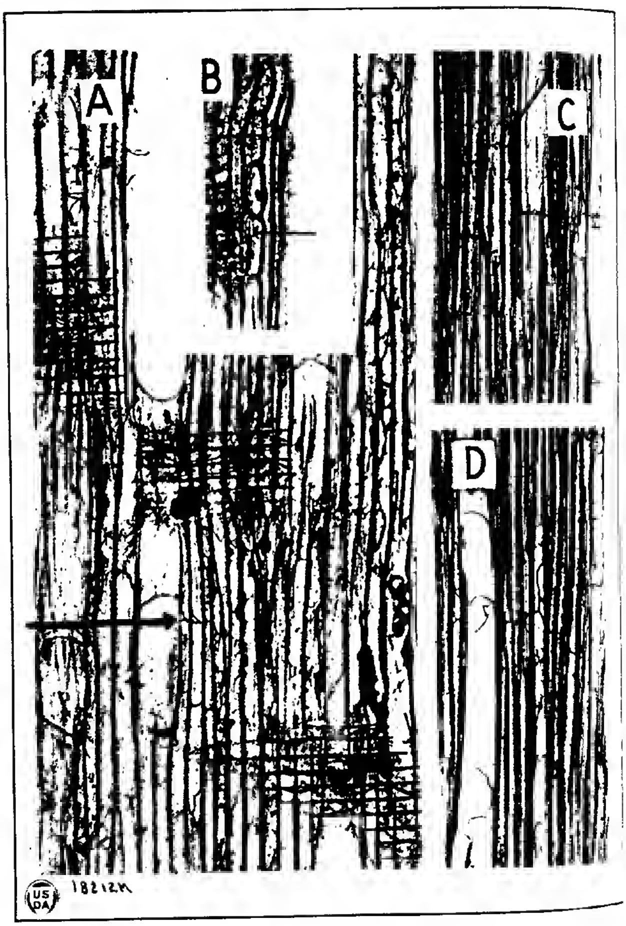
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PLATE 4

A.—Aspen (radial section) attached by *Penicillium divaricatum.* Note mycelium penetrating cell wall (arrow near center), traversing ray and fiber cavities. The colorless mycelium of different sizes is differentiated from the host tissue in each case by soaking the section for some time in a dilute solution of gold chlorid in distilled water.

B.—A white oak inoculated with *Penicillium divaricatum.* The fungous mycelium is to be seen in its course through a group of parenchyma cells. Stained with dilute gold chlorid from 8 a. m. to 4 p. m.

C and D.—Aspen, same as A, but in tangential section.

OMMON EARTHENWARE JARS A SOURCE OF ERROR IN POT EXPERIMENTS[1]

By J. S. McHargue

esearch Chemist, Department of Chemistry, Kentucky Agricultural Experiment Station

In an investigation to determine whether or not manganese is necesary for the normal growth of plants, by means of carefully prepared pot ultures, occasional results were obtained in the control pots which ndicated that the plants were obtaining manganese from an unrecogized source. Since manganese had been carefully eliminated from the and and the mineral nutrients mixed with it, it was evident that the ot was the source of manganese, although the pot was clean and apparntly well glazed on the inside surface at the time the nutrients were dded.

It had been observed previously that among the 80 pots in use in this xperiment there were a few on which crystalline deposits of mineral utrients appeared on the outside after they had been wet a few times. his fact showed that the walls of the pots were porous and not suffiiently well glazed to prevent the migration of moisture which carried e mineral nutrients in solution through the walls so that subsequent vaporation and deposition of the mineral nutrients occurred on the utside. Judging from external appearances, these pots were as well lazed as other pots on the outside of which no deposit of mineral nutrints occurred.

In Plate 1, the only plate accompanying this article, and in references o which only the letters A, B, and C will be used, A shows the extent f the migration and deposition of the mineral nutrients through the alls of the pot. The white, frosted material which appears plainly on he brown glaze extended practically over the outside surface of the pot. ots similar in grade to those shown in A and C are in common use in ot experiments at agricultural experiment stations.

The observation that a few of the total number of pots were sufficiently orous to allow mineral nutrients to migrate through their walls suggested e idea that other similar pots might have walls sufficiently porous to bsorb, from soils or sand used in culture experiments conducted in em, nutrients which would affect the results of other experiments ade in the same pots at a later time.

This conjecture is supported by results obtained in experiments with anganese. Tomato plants were grown in pots that had been previsly used in other experiments and were similar in grade to the pot own in A. No deposit of mineral nutrients occurred on the exterior f any of these pots when like amounts and kinds of mineral nutrients ere mixed with the sand in the several pots.

C represents two of these pots containing tomato plants that were rown in purified sand and mineral nutrients. Manganese was carefully xcluded from the sand culture on the left, whereas the one on the right ontained 0.25 per cent of manganese in the form of the carbonate. he sand cultures were kept at the proper moisture content by frequent

[1] Accepted for publication July 11, 1923.

weighings and the addition of distilled water during the time the plants were making their growth. The plants on the right represent a slightly more vigorous growth than those on the left. The plants on the left differed most from those on the right by the branches and leaves at the top becoming chlorotic a short time before the photograph for C was made, whereas those on the right maintained a normal green color. While the chlorotic condition of the plants on the left is characteristic of the lack of manganese, this condition was expected to occur at a much earlier time in the growth, unless the plants received manganese from the pot.

To prove that the pot was a source of manganese, new pots were made of acid-proof stoneware and the experiment with tomato plants was repeated. The result is shown in B.

The difference in the growth of the tomato plants in the pots on the left in B and C is due to the fact that the pot on the left in C contained manganese absorbed in the walls of the pot, and this became available to the plants during the earlier part of their growth. Apparently the supply of manganese became exhausted a short time before this photograph was made, as is indicated by the fact that the branches and leaves became chlorotic and showed other signs characteristic of plants deprived of the amount of manganese necessary for their growth.

The plants in the pot on the left in B illustrate the condition attained when manganese is entirely eliminated from a sand culture containing available compounds of the 10 elements which have hitherto been regarded as all that are necessary for the growth of plants. The plants in the pot on the right in B grew in sand containing the same amount of these compounds and enough manganese carbonate to supply about 0.25 per cent of the element manganese, to the sand. The plants in the two pots are of the same age.

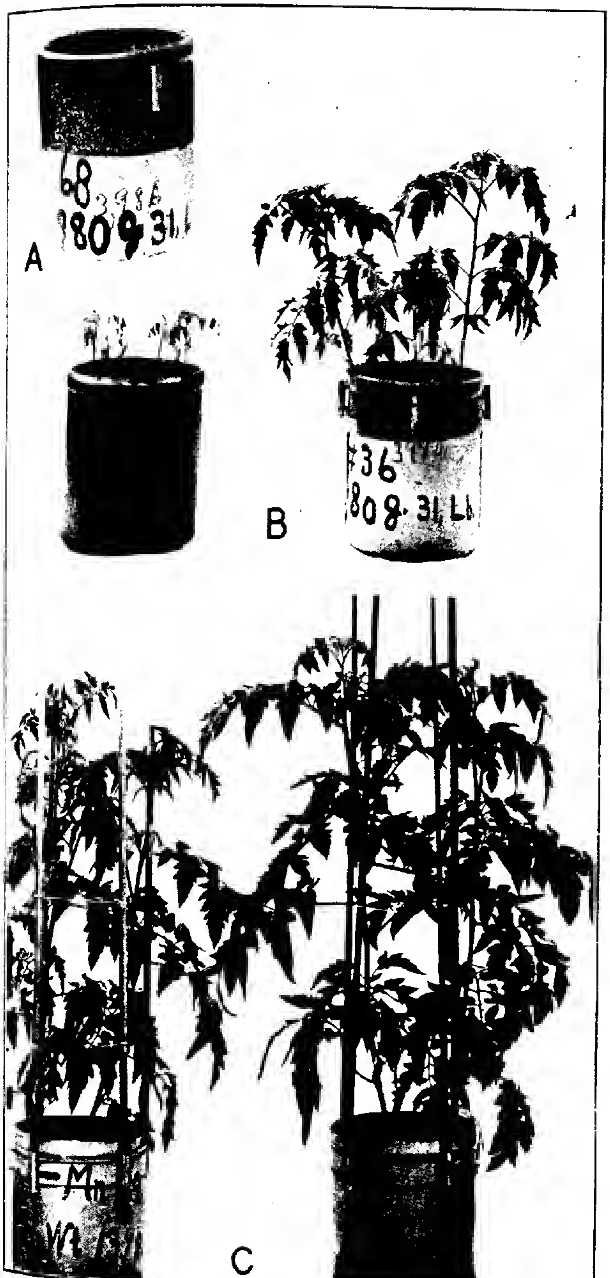
From the facts here presented it seems evident that earthenware pots of the grade in common use in pot experiments may be sufficiently porous to absorb enough plant nutrients to affect the growth of other plants grown in the same pots at a later time. Acid-proof stoneware should be used in exact work.

PLATE 1

A.—The frosted effect on the surface of the pot is due to mineral nutrients migrating through the walls.

B.—The illustration on the left shows the effect produced when tomato plants are grown in a pot made from acid-proof stoneware containing a sand culture free of manganese but with the same quantity and kind of other plant nutrients as the pot on the right.

C.—The plants on the left obtained manganese from the pot. Compare with the plants on the left in B.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THE PHYSIOLOGICAL EFFECT OF GOSSYPOL [1]

By Paul Menaul [2]

Department of Chemistry, Oklahoma Agricultural Experiment Station

Since the value of cottonseed and cottonseed meal as feedstuffs has become so widely recognized, numerous investigations have been made to determine the nature of the toxic substance contained in these products. Various suggestions, summarized elsewhere (*1, 2, 5, 6, 8*),[3] have been made as to the cause of poisoning and death resulting from the use of cottonseed and cottonseed meal as feedstuffs. Withers and Carruth (*9, 10*) have shown that the poisonous property of the cottonseed is due to a phenolic substance called "gossypol," first isolated by Marchlewski in 1899.

The effect of gossypol poisoning on several species of animals is shown in the experiments recorded in this article. The gossypol used was prepared by crystallization from acetic acid, and was dissolved for use in N/10 sodium hydroxid, any excess alkali being neutralized with acid so that the solution was neutral to litmus.

One-half gm. of gossypol administered orally produced no serious effects on a rabbit weighing 4 pounds. The rabbit ceased eating, but no symptoms of poison were noted. One-half gm. of gossypol injected intraperitoneally produced no abnormal symptoms for 36 hours, although the animal refused food during this time and on the fourth day thereafter died. One-tenth gm. of gossypol injected into the marginal vein of a rabbit weighing 4 pounds caused death in about four minutes. The animal acted as though it were being suffocated, leaping high into the air and gasping. Five-hundredths gm. was given to another rabbit in the same manner. In 10 minutes it became very weak and lay on the floor, unable to move its limbs. Within an hour it had recovered the use of its limbs and sat up, but 16 hours later it died, having developed hemoglobinuria. Continued feeding of small amounts of gossypol, 0.1 gm. per day, to each of four rabbits resulted in intestinal inflammation. The rabbits died about 14 days after the feeding of gossypol was begun.

EFFECT OF GOSSYPOL ON HEMOGLOBIN ABSORPTION SPECTRA

One-half cc. of washed blood corpuscles in 75 cc. of water were examined with the spectroscope. The two absorption bands near the "D" line were very clear and distinct. One-hundredth, three-hundredths, and six-hundredths gm., successively, of gossypol in 1 cc. of solution were added, but no change in the two lines near "D" could be detected. There was no evidence that the oxyhemoglobin had been reduced. Since the solutions of gossypol are slightly yellow, the addition of gossypol to hemoglobin solutions causes more of the blue in the spectra to be absorbed.

[1] Accepted for publication July 18, 1923.

[2] This study was undertaken at the suggestion of Dr. C. T. Dowell, director of the station and station chemist. I wish to acknowledge my indebtedness to him for his sympathetic cooperation.

[3] Reference is made by number (italic) to "Literature cited," p. 237.

EFFECT OF GOSSYPOL ON THE OXYGEN CAPACITY OF THE BLOOD

Fresh sheep's blood was centrifuged to concentrate the corpuscles; these were saturated with oxygen and used in the following experiments. The "oxygen capacity" was determined according to the method of Van Slyke (7). Determinations were made using the concentrated blood corpuscles, and also using whole blood. In each case 1 cc. of a 1 per cent NaCl solution was added to 2 cc. of the corpuscles or of whole blood, the mixture placed in the apparatus, and the oxygen liberated determined; two such determinations were made as a control in each series of experiments. Then a similar mixture was made of 2 cc. of blood corpuscles or of whole blood, and 1 cc. of 1 per cent NaCl solution containing a definite amount of gossypol, and the oxygen liberated determined as before.

For the first set of comparisons, the two determinations with blood corpuscles gave as results 0.75 and 0.745 cc. of oxygen; mean of the two, 0.7475. A similar determination, using NaCl solution which contained 0.02 gm. of gossypol, yielded 0.35 cc. of oxygen, or only 46.8 per cent of the mean of the two control determinations. With 0.0025 gm. gossypol, 0.48 cc. of oxygen was liberated, or 64.2 per cent.

Two further determinations of the oxygen content of similar mixtures of blood corpuscles and NaCl solution gave 0.745 and 0.75 cc. of oxygen; mean, 0.7475, as before. With 0.01 gm. of gossypol in the 1 cc. of NaCl solution used, 0.48 cc. of oxygen were liberated; two further determinations, each with 0.01 gm. of gossypol, gave 0.45 and 0.46 cc., respectively, of oxygen; mean of the three, 0.463, or 61.9 per cent of the oxygen liberated with no gossypol present.

Again, the two control determinations, with the usual mixture of blood corpuscles and NaCl solution, gave 0.64 cc. and 0.63 cc. of oxygen; mean, 0.635. Three successive determinations, each with 0.004 gm. of gossypol contained in the 1 cc. of NaCl solution, yielded 0.26 cc., 0.30 cc., and 0.30 cc., respectively, of oxygen; mean of the three, 0.287, or 45.2 per cent of the oxygen liberated from the mixture free of gossypol.

A series of determinations was also made with a mixture of 2 cc. of whole blood and 1 cc. of the usual 1 per cent NaCl solution. Two control determinations gave 0.43 cc. and 0.435 cc. of oxygen; mean, 0.4325. Two determinations were then made with a similar mixture, the 1 cc. of NaCl solution of which contained in each case 0.005 gm. of gossypol. Each determination gave 0.32 cc. of oxygen, or 74.0 per cent of the mean value of the control determinations. And, finally, two similar determinations, with double the amount of gossypol, or 0.01 gm., in each mixture, liberated 0.27 and 0.28 cc. of oxygen; mean, 0.275, or 63.6 per cent of the oxygen liberated from the mixture free from gossypol.

It is clear from the results here recorded that gossypol inhibits the liberation of oxygen from hemoglobin. This property of gossypol is evident even when very small quantities are used. The results are such as might have been anticipated from the symptoms observed in animals suffering from gossypol poisoning—namely, a shortness of breath following muscular exertion.

HEMOLYTIC ACTION OF GOSSYPOL

Gossypol dissolves in dilute alkaline solutions, thereby neutralizing hem. If such solutions are shaken a thick foam is formed as in the case f saponins. The hemolytic power was determined on sheep's blood. 'he blood was washed three times by centrifuging. The corpuscles were hen suspended in suitable concentrations in physiological salt solution. 'he experiment was conducted at room temperature, 20°C. Twenty-our cc. of diluted corpuscles were put into 30 cc. tubes and 1 cc. of a olution containing a varying quantity of gossypol in 0.6 per cent NaCl 'as added, the contents of the tube mixed, and the time of complete emolysis noted. The concentration of the corpuscles and the results btained are given in the following table:

I.—*Blood corpuscles diluted 1 cc. in 96 cc.*

Tube No.	Gossypol added.	Gossypol in tube.	Approximate time of complete hemolysis.
	Gm.	*Per cent.*	
....	0.025	0.1	10 seconds.
....	.0125	.05	20 seconds.
....	.005	.02	30 seconds.
....	.0025	.01	15 minutes.
....	.00125	.005	Only slight hemolysis noted in 3 hours.
....	.00	.00	Unchanged in 5 hours.

II.—*Blood corpuscles diluted 1 cc. in 24 cc.*

Tube No.	Gossypol added.	Gossypol in tube.	Approximate time of complete hemolysis.
	Gm.	*Per cent.*	
....	0.025	0.1	10 seconds.
....	.0125	.05	20 seconds.
....	.0005	.02	35 seconds.
....	.0025	.01	Incomplete in 2 hours.
....	.00125	.005	Unchanged in 3 hours.
....	.000	.000	Unchanged in 5 hours.

EFFECT OF GOSSYPOL ON FISH

Perch about 2 inches long were used in the following group of experi-ients. For each observation two fish were placed in a large jar contain-ig 5 liters of the gossypol solution. The controls showed no sign of xygen deficiency after nine hours.

Experiment No.	Amount of gossypol.	Dilution of gossypol.	Remarks.
	Gm.		
....	0.1	1:50,000	Both fish died in 45 minutes. Before death fish rose often to the surface and gasped.
....	.1	1:50,000	Same as No. 1. Air bubbled through water had no effect.
....	.05	1:100,000	Both fish died in 1¼ hours.
....	.05	1:100,000	Same as No. 3. Air bubbled through the water had no effect.

Experiments 2 and 4 indicate that death was not due to a lack of dissolved oxygen in the water.

The following experiments were made in duplicate and identical results were obtained in each case.

5. One-tenth gm. of gossypol, 25 cc. H_2O_2 and 20 gm. of ether-extracted, unheated cottonseed meal were mixed and added to 5 liters of water in which two fish had been placed. The fish remained normal for nine hours.

6. One-tenth gm. of gossypol and 25 cc. of H_2O_2 were added to 5 liters of water and two fish were placed in the solution. The fish died in 1½ hours, as in experiments 3 and 4.

7. One-tenth gm. of gossypol, 25 cc. H_2O_2 and 20 gm. of ether-extracted "hot-pressed" cottonseed meal were mixed and added to 5 liters of water, and two fish were dropped into the liquid. The fish died in 1½ hours, as in experiments 3, 4, and 6.

Gossypol is toxic to fish as a dilution of 1:100,000; hydrogen peroxid does not destroy its toxicity when in solution. Hydrogen peroxid, in conjunction with unheated cottonseed meal, destroys the toxicity of gossypol when in solution, probably through the agency of a peroxidase enzym.

ANALYSIS OF THE BLOOD AND URINE OF ADULT SHEEP ON A DIET OF COTTONSEED MEAL

An adult male sheep was fed 1 pound of cottonseed meal per day beginning April 10. The sheep was kept on green pasture except during the days when it was confined in a metabolism cage for the collection of the urine. Samples of blood and urine were collected at intervals, and upon analysis gave the results shown in the following table. The system of blood analysis by Folin and Wu (*3*) was followed for the determination of the blood constituents, and the methods outlined in Hawk's Practical Physiological Chemistry (*4*) were used for the analysis of the urine.

BLOOD CONSTITUENTS

	Apr. 4.	Apr. 11.	May 9.	May 18.	May 25.	June 2.	June 8.	June 15.	June 24.	Ju[illegible] 30
Non-protein N in mgm. per 100 cc. blood	32.4	33	46.02	44.43	45	44	41.3	31.4	30	[illegible]
Sugar (per cent)	.069	.071	.087	.084	.086	.087	.088	.064	.049	[illegible]

URINE CONSTITUENTS

	Apr. 16.	May 2.	May 6.	May 15.	May 21.	May 24.	May 31.	June 3.	June 20.	Ju[illegible]
Volume in cc.	500	1,250	1,910	2,350	1,600	2,100	1,260	1,800	1,550	[illegible]
Specific gravity	1.026	1.032	1.026	1.02	1.017	1.023	1.033	1.030	1.0325	[illegible]
Total N in gm.	5.92	19.09	22.22	23.25	28.0	23.85	22.38	30.0	26.26	[illegible]
Urea N in gm.	3.506	12.24	16.39	17.36	......	16.93	17.14	24.12	19.05	[illegible]
Ammonia N in gm.	1.33	1.34	1.05	1.071	......	2.99	2.04	2.07	3.33	[illegible]
Creatinin N in gm.	1.2	1.21	1.24	1.07	......	1.2	1.5	1.3	1.3	[illegible]
Total acetone bodies in gm.	.25	1.2	......	......	2	......	3	2.1	1.5	[illegible]

Cottonseed meal is here shown to have a diuretic action. At first the concentration of the nonprotein nitrogen and sugar of the blood are increased, but after the second month they are lowered far below t

rmal. The urine constituents show the result of a high protein diet d also the development of slight acidosis.

CONCLUSIONS

By experiments on rabbits, gossypol is shown to be absorbed slowly ıen administered through the diet, and its toxic action is slow to ake its appearance. When introduced directly into the blood stream ; toxic action is manifest at once. Its most serious effect is on the ood. By determining the amount of oxygen that can be liberated om blood before and after the addition of small quantities of gossypol, is clear that in some manner the gossypol prevents the liberation of e oxygen from oxyhemoglobin. Gossypol also exerts a hemolytic ect on the erythrocytes.

Gossypol causes death in animals by reducing the oxygen-carrying pacity of the blood. Thus an excessive burden is thrown on the spiratory and circulatory organs which results in the condition found animals that have died from gossypol or cottonseed meal poisoning—mely, a passive hyperemia and oedema of the lungs and some hydro-orax. These conditions are always present and are not due to bac-rial infection.

LITERATURE CITED

ALSBERG, C. L., and SCHWARTZE, E. W.

1919. PHARMACOLOGICAL ACTION OF GOSSYPOL. (Abstract). *In* Jour. Pharm. and Experimental Ther., v 13, p. 504.

CARRUTH, Frank E.

1918. CONTRIBUTION TO THE CHEMISTRY OF GOSSYPOL, THE TOXIC PRINCIPLE OF COTTONSEED. *In* Jour. Amer. Chem. Soc., v 40, p 647–663. Bibliographical footnotes.

FOLIN, Otto, and WU, Hsien.

1919. A SYSTEM OF BLOOD ANALYSIS. *In* Jour. Biol. Chem., v. 38, p. 81–110, 2 fig. Bibliographical footnotes.

HAWK, Philip B.

1921. PRACTICAL PHYSIOLOGICAL CHEMISTRY. Ed. 7. xiv, 675 p., illus. Philadelphia.

OSBORNE, THOMAS B., and MENDEL, Lafayette B.

1917. THE USE OF COTTONSEED AS FOOD. *In* Jour. Biol. Chem., v. 29, p. 289–317, 5 charts.

RICHARDSON, Anna E., and GREEN, Helen S.

1916. NUTRITION INVESTIGATIONS UPON COTTON SEED MEAL. I. *In* Jour. Biol. Chem., v. 25, p. 307–318, 5 charts. Bibliographical footnotes.

VAN SLYKE, Donald D.

1918. GASOMETRIC DETERMINATION OF THE OXYGEN AND HEMOGLOBIN OF BLOOD. *In* Jour. Biol. Chem., v. 33, p. 127–132.

WELLS, C. A., and EWING, P. V.

1916. COTTONSEED MEAL AS AN INCOMPLETE FOOD. *In* Jour. Biol. Chem., v. 27, p. 15–25. References, p, 24–25.

WITHERS, W. A., and CARRUTH, F. E.

1915. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTON SEED MEAL. *In* Jour. Agr. Research, v. 5, p. 261–288, pl. 25–26. Literature cited, p. 287–288.

———

1918. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED, *In* Jour. Agr. Research, v 12, p. 83–102, 3 fig., pl. 1. Literature cited, p. 100–101.

IRON CONTENT OF THE BLOOD AND SPLEEN IN INFECTIOUS EQUINE ANEMIA [1]

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Very little is known of the iron content of the blood or organs in fectious equine anemia, other than the changes in the blood that late to the clinical hemoglobin estimation. While the writer was work-g on the problem of infectious equine anemia as a whole this study as undertaken. At the beginning of the investigation two problems esented themselves. One was found in the fact that in the examina-on of the blood in this disease there was often a fairly high erythrocyte unt with a low hemoglobin percentage, together with many shadow rpuscles found in the smears, seeming to show a greater loss of hemo-obin than the erythrocyte count in itself would indicate. For this ason the determinations on the blood were made. The second prob-n was to determine the fate of the cells after destruction, if the anemia due to an increased destruction of red cells. In this disease it is ex-ptional to find any marked loss of blood or hemoglobin from the body ough any of the body discharges, as the urine or feces, nor does mination of the urine disclose any marked evidence of an increased pigment elimination. There might of course be elimination through feces, but these have not been examined for iron. Since the spleen nown to be a seat of erythrocyte destruction, the idea was suggested t possibly there was unusual destruction of red cells in the spleen h retention of the iron. For this reason the splenic determinations e made.

ections from the liver and spleen when properly fixed and stained e shown large amounts of an iron-containing pigment, probably osiderin. Because of this it would have been advisable to make erminations on the liver also. As stated above, iron elimination uld also be studied, for this may be one of the most important phases he whole problem of anemia. In this, as in other anemic conditions, not impossible that one factor in its course is a lack of available for the formation of red cells. Increased iron elimination might se this.

he determinations given in this article are far too few for one to at-pt to make any positive deductions from them, but they are certainly gestive and are published for what they are worth. The fact that writer will not have an opportunity to continue this study accounts the incompleteness of the data here presented.

he blood used in the determinations was drawn from the jugular , collected in a test tube, taken to the laboratory at once, and the ple weighed before there was any chance for loss by evaporation. spleen was taken at the autopsy, which was made as soon after th as possible. A small portion of the spleen was cut off, put into a

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[2] signed June 1, 1920.

jar, and at once taken to the laboratory, where it was weighed. The iron content was determined on air-dry material. The loss in drying was noted, and the iron found was calculated to parts of Fe_2O_3 per thous. and parts of fresh sample. The iron was determined on 1-gm. samples by the iodometric method of A. Neumann [3] after destruction of the organic matter by digestion with a mixture of sulphuric acid and nitric acid. Care was necessarily taken to use reagents that were free from iron, and samples were at no time exposed to contamination by metallic iron or its salts. The total red counts were made in the usual manner, the ordinary precautions being observed. The blood was procured from the under surface of the tail. The hemoglobin was determined by the Talquist method.

TABLE I.—*Iron content of horses' spleen in infectious equine anemia*

No.	Description of animal.	Date.	Spleen weight.	Solids, air-dry parts per 1,000.	Fe_2O_3 fresh parts per 1,000.	Total Fe_2O_3
			Gm.			*Gm.*
17	Young, normal; shot	Mar. 2	910	220.6	0.29	[illegible]
9	Aged, normal; shot	Mar. 18	1,240	238.6	5.59	[illegible]
2	Aged, normal; shot	May 27	1,025	253.3	4.23	[illegible]
22	Young, bled 9½ liters, Aug. 8; shot	Aug. 12	787	244.0	.48	[illegible]
20	Young, bled 10 liters, Aug. 8; shot	Aug. 14	910	234.5	.28	[illegible]
6	Young, acute infectious equine anemia; died	Apr. 16	6,257	250.0	1.18	[illegible]
21	Aged, acute infectious equine anemia; died	Sept. 29	5,233	260.0	1.08	[illegible]
26	Aged, acute infectious equine anemia; died	Feb. 23	5,119	271.4	1.45	[illegible]
753	Young, chronic infectious equine anemia; shot	Feb. 26	1,025	228.9	.43	[illegible]
23	Aged, chronic infectious equine anemia; shot	Feb. 25	1,934	222.0	2.48	[illegible]
18	Aged, chronic infectious equine anemia; shot	Feb. 10	1,365	236.1	1.58	[illegible]
25	Aged, chronic infectious equine anemia; shot	Mar. 18	1 365	222.3	4.44	[illegible]
	Averages:					
	Acute infectious equine anemia		5,536	260.4	1.23	[illegible]
	Bled		848.5	239.2	.38	[illegible]
	Normal		1,058	237.5	3.37	[illegible]
	Chronic infectious equine anemia		1,422.2	227.5	2.23	[illegible]
	Normal aged		1,132.5	245.9	4.91	[illegible]
	Normal young		910	220.6	.29	[illegible]
	Total aged		2,468.7	243.5	2.98	[illegible]
	Total young		1,997.8	235.6	.53	[illegible]

As has been previously stated, this study is based on a very small number of cases and, therefore, any statements made must be guarded. However, the results of these determinations on the blood and spleen

[3] NEUMANN, ALBERT. UEBER EINE EINFACHE METHODE DER EISENBESTIMMUNG BEI STOFFWECHSELVERSUCHEN. 2. MITTHEILUNG. *In* Arch. Anat. u. Physiol., Physiol. Abt., 1902, p. 362-365. 1902.

om normal horses are in accord with those of other observers. It is interest to note the enormous increase in iron in the spleens of old imals over that in young ones. A study of Table I indicates that the iantity of iron is greatly increased in the spleens of young horses suffering om acute infectious equine anemia, a condition which would naturally llow from the great destruction of red cells. Such a statement does ot hold true, however, for old horses or chronic cases. The spleens om old animals with chronic anemia usually show less iron than those om old normal horses. The weight of the spleens from the acute ses is nearly five times as great as those from normal animals or aniials with chronic anemia. It is worthy of note that the old horses ffering from the disease in an acute form had greatly enlarged spleens, it the total iron content was only slightly greater than in the old imals that were normal. This is just contrary to the results found No. 6. There seems to be no increase in iron in the spleens of young imals with chronic cases. As a general observation, the evidence of iemia is less marked in the chronic cases than in the acute ones.

TABLE II.—*Iron content of horses' blood in infectious equine anemia*

Condition of animal at time of taking blood.	Date.	Solids, air-dry parts per 1,000.	Fe_2O_3 in fresh parts per 1,000.	Hemoglobin.	Erythrocytes.
				Per cent.	
Normal	Apr. 11	196.0	0.59	90	7,964,000
....do	May 27	215.0	.63	100	8,032,000
....do	Apr. 11	200.0	.59	90	7,244,000
....do	May 26	204.6	.55	90	7,648,000
....do	Apr. 11	197.4	.65	90	7,860,000
Sick	..do....	211.1	.45	90	6,836,000
Normal	May 29	231.6	.76	100	7,964,000
....do	June 4	251.2	.86	100	7,856,000
Sick	Aug. 26	193.7	.45	80	7,288,000
....do	Sept. 12	195.3	.53	80	6,972,000
Normal	Nov. 1	230.7	.55	90	8,262,000
Normal, bled 10 liters Aug. 8.	Aug. 14	182.4	.51	....	....
Normal	June 9	188.5	.44	80	6,974,000
....do	Aug. 12	200.1	.51	....	....
Sick	Sept. 12	158.4	.36	80	6,464,000
....do	Sept. 18	153.4	.30	....	....
....do	Sept. 30	147.9	.33	....	....
Normal, bled 9½ liters Aug. 8.	Aug. 12	149.8	.35	....	....
Normal	Aug. 26	205.9	.59	90	7,844,000
Sick	Sept. 12	178.8	.36	70	6,864,000
....do	Nov. 1	182.5	.40	80	6,824,000
Normal	Sept. 18	179.1	.42	90	6,988,000
....do	Nov. 1	181.5	.46	80	6,464,000
Sick	Nov. 14	227.6	.53	....	....
....do	Apr. 11	146.7	.23	....	....
Normal	June 9	200.7	.59	100	7,764,000
....do	Aug. 12	190.2	.49	....	....
....do	Sept. 12	186.1	.50	90	7,688,000
Very ill	June 19	117.7	.14	30	3,486,000
Normal	June 4	218.8	.63	....	....
Averages:					
Normal	....	204.6	.577	91	7,622,000
Sick	....	173.9	.371	73	6,371,000
Bled	....	166.1	.340	....	....

The averages from Table II show what one might expect, that is, that the average totals of the solids—iron, hemoglobin, and erythrocytes—are greater in the normal animals than in the sick ones. There is more actual anemia due to a lack of iron, and therefore a deficiency of hemoglobin, than the total erythrocytes would indicate. This also would be expected from the large number of shadow corpuscles which are often found in cases of anemia.

The increased iron content of the spleens of the young animals can not be due solely to the increased quantity of blood in the organ, for if the extra weight of the spleen were due wholly to the weight of blood the additional iron would not be sufficient to account for the increase. A study of the tables substantiates this statement.[4]

[4] The writer takes this occasion to express his gratitude to Prof. M. R. Miller for valuable help and advice in carrying out this investigation.

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